



# POLIOMYELITIS

*Papers and Discussions Presented at the  
Fourth International Poliomyelitis Conference*

Compiled and Edited for the  
INTERNATIONAL POLIOMYELITIS CONGRESS



Philadelphia

Montreal

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### *Editorial Note*

*The material in this volume comprises a record of the Fourth International Poliomyelitis Conference including a report of the opening session the banquet and the other functions The original presentations have been included in toto The discussions addresses and exhibits have been edited carefully and specially prepared for publication All the material delivered in several languages and transcribed by recording has been edited in the interest of accuracy and brevity*

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## *Dedication*

Previous volumes of the proceedings of the International Poliomyelitis Conference have been dedicated to Franklin Delano Roosevelt whose leadership and inspiration gave birth to the Georgia Warm Springs Foundation the National Foundation for Infantile Paralysis and the International Poliomyelitis Congress This book is again dedicated to Franklin Delano Roosevelt

The Fourth International Poliomyelitis Conference was concerned primarily with the preventive vaccine now in use throughout the world which seems likely to terminate such epidemics of infantile paralysis as have threatened mankind in the past

This volume is dedicated also to Professor Edmond Grasset distinguished scientist in the field of public health and preventive medicine who for three years served as chairman of the committee which had charge of arrangements for the Fourth International Poliomyelitis Conference He died in September 1957 His untiring efforts his kindness his leadership his knowledge and his special ability in securing co-operation from the representatives of many different nations will never be forgotten by those who had the privilege of working with him

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BASIL O CONNOR





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## *Preface*

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The First International Poliomyelitis Conference was held in 1948 in New York City the Second in Copenhagen in 1951 the Third took place in Rome in 1954 So great has been the interest in the published proceedings of these conferences that the proceedings of the Fourth Conference held in Geneva Switzerland 1957 are now published in similar form

Copies of the first three volumes are available in practically all the medical libraries in the world Thousands of copies have been circulated to individual investigator and to institutions for medical research

The proceedings of the Fourth Conference include discussions on many different phases of poliomyelitis but the centers of interest were vaccination against the disease new information concerning enteric viruses that produce diseases simulating poliomyelitis and general considerations of viruses and of cultures of mammalian cells New information was made available concerning new techniques in the diagnosis of infantile paralysis Other sessions were devoted to group and home care of patients with respiratory or extensive paralysis and the care of patients severely stricken by the disease The scientific exhibit was an important feature of the sessions and other aspects including the social functions are also covered by these proceedings

The editor and the publisher again extend their thanks to the contributors and to the educational institutions which collaborated in making possible the publication of the proceedings

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# Reports of Official Delegates

MONDAY MORNING JULY 8 1957

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*AN OUTLINE OF RED CROSS  
ACTIVITIES IN THE FIGHT  
AGAINST POLIOMYELITIS*

DR Z S HANTCHEF

THE RED CROSS AND THE FIGHT  
AGAINST POLIOMYELITIS

At the basis of all activities undertaken by National Red Cross societies is the diffusion among the population of humanitarian principles and respectively the application of these principles in the prevention and the relief of man suffering

For this reason the Board of Governors the highest authority and the General Assembly of

League of Red Cross Societies considering it in recent years poliomyelitis has become a problem of ever increasing importance in all parts of the world adopted the recommendations made by the League's Health Advisory Committee and advised all national societies to participate as actively as possible in the fight against this disease

At the 23rd session held at Oslo in 1954 the Board of Governors of the League

recommends that National Societies should include and stress in their health programmes the precautions and measures which could be taken generally and especially in times when poliomyelitis is prevalent. These are all points which may easily with some appropriate explanation be included not only in any Red Cross health education programme for the general public but also for the programmes of the Junior Red Cross. As it is obvious that passive immunisation (such as for instance gamma globulin offering only temporary protection) has only a limited use active immunisation constitutes the final answer to the problem of protection against poliomyelitis. Therefore the Board of Governors is of the opinion that no Red Cross Society should undertake the produc-

tion of gamma globulin as a method of prevention of poliomyelitis only and that should any method of active immunisation be developed as may reasonably be expected Red Cross Societies should be encouraged to assist their governments as far as possible in carrying out their vaccination programmes

Considering that the need for qualified nursing personnel constitutes a major problem during acute outbreaks of poliomyelitis

and that equally important is the problem of providing auxiliary personnel to give artificial respiration to the many patients suffering from respiratory paralysis during their transportation to and their stay in hospital

the Board of Governors advises Red Cross Societies to train auxiliary personnel to cover this emergency and whereas in small communities sharp outbreaks are perhaps best dealt with by mobile teams organised on a regional level based on the principle of mutual assistance between sister Societies

recommends that as this task may exceed the powers of the Red Cross collaboration with other international health organisations working in this field such as the World Health Organisation should be encouraged and

whereas besides the special medical problems within the rehabilitation in poliomyelitis there are important social problems and these are from a Red Cross point of view the most important

considering that the importance of social assistance to chronic poliomyelitis patients can hardly be overestimated

advises therefore the National Societies to establish a close collaboration in this field with the public health authorities and the national associations against poliomyelitis in the respective countries

considering that only by supporting to the full extent of its powers the common fight against this social menace can the Red Cross be said to fulfil one of its fundamental tasks the prevention and alleviation of the suffering of humanity\*

Resolution No XIV of the Board of Governors 1954



Following up this resolution national societies have intensified their efforts in the fight against poliomyelitis and have been assisted by the League Secretariat and more particularly by the Medico Social Bureau. The latter has as its task the co-ordination of medico-social activities of the various national societies and the liaison with different international organizations such as the World Health Organization (WHO).

On two occasions—first the 23rd session of the Board of Governors in 1954 and second the Fourth International Poliomyelitis Conference in 1957—national societies have sent to the League reports on their activities in the fight against poliomyelitis. We have summarized these reports as follows:

#### OUTLINE OF ACTION TAKEN BY NATIONAL SOCIETIES

**American Red Cross.** All agencies involved in poliomyelitis care recognize that the responsibility for epidemic control is that of governmental health agencies. A poliomyelitis planning committee under the direction of the state health officer functions in many of the states. The American Red Cross and other agencies concerned with the care of the poliomyelitis patient are represented on this committee.

In poliomyelitis epidemics the total volunteer resources of the Red Cross are available to help meet the needs in the community. Volunteers transport nurses and family members to the hospital, take convalescent patients to clinics and maintain a system of reporting so that families can be notified daily of the condition of the hospitalized patients. Junior Red Cross members furnish books, toys and other recreational materials for children. An undetermined number of volunteer nurse's aides have been assigned to assist professional nurses in the care of poliomyelitis patients.

Since 1943 the American Red Cross has been co-operating closely with the National Foundation for Infantile Paralysis and formal agreements have since been drawn up between the two organizations. Thus it was that the American Red Cross agreed to act as the recruiting agency for the National Foundation for Infantile Paralysis and has since 1949 co-operated with the National League for Nursing in planning in-service training programs for recruited nurses.

Each local Red Cross committee maintains a list of nurses who agree to serve for a 3 month period if needed. Many of these nurses assist health departments in giving inoculations of gamma globulin or Salk vaccine on a volunteer basis.

Furthermore it is to be noted that the American Red Cross has co-operated with the National Foundation for Infantile Paralysis for the supply of gamma globulin from its Blood Transfusion Service.

**Australian Red Cross.** The State Department of Public Health has asked the central Red Cross Blood Transfusion Service to undertake the cleaning and the sterilizing of the equipment used in the country's mass vaccination campaign. It is estimated that 7 000 needles and 700 syringes will be used each day. The State Department considers that there is no other organization which could undertake this work on such a vast scale. All costs for the service will be borne by the Health Department. As usual Red Cross workers will assist councils and local bodies during the course of the campaign.

**Canadian Red Cross.** In the years preceding the discovery of the Salk vaccine the Canadian Red Cross carried through an extensive program in the collection of blood and the manufacture and the distribution of gamma globulin. This field of activity was developed in agreement with the Provincial Departments of Health and incorporated many staff and volunteer workers.

At the Alberta Red Cross Crippled Children's Hospital 473 poliomyelitis cases have been treated since January 1952. The staff of the hospital is assisted by numerous regular and part-time volunteers. In view of the marked decrease in poliomyelitis and surgical tuberculosis the building and the equipment have been turned over to a local agency to be operated as a general hospital for children.

The Canadian Red Cross has also a water therapy program and 3 curative workshops for physiotherapy. Finally there is the Junior Red Cross Handicapped and Crippled Children's Fund through which needy children handicapped in any way are helped with the cost of treatment, hospitalization, orthopedic appliances, etc.

**Czechoslovakian Red Cross.** On the request of the public health authorities this society

will participate in a vaccination campaign for 2 000 000 children. The Czechoslovak Government bought from Canada all the vaccine necessary for this large scale operation.

**Danish Red Cross.** This society is co-operating closely with another organization the Society and Home for Cripples. According to an agreement between the two bodies polio patients are being received at the Danish Red Cross Convalescent and Rehabilitation Center at Hald near Viborg (Jutland) and are being treated by doctors employed by the above mentioned organization. At the Hald Center there is a modern pool for the treatment of polio patients; this was built with the support of the Danish Polio Association with which the Danish Red Cross is also in co-operation.

**Ecuadorian Red Cross.** Because of the small number of poliomyelitis cases in Ecuador this society has concentrated its efforts on the supplying of orthopedic appliances. Moreover it is leading a movement promoting the giving of Salk vaccination to the population.

**Swedish Red Cross.** In 1949 the Royal Board of Public Health contacted the Swedish Red Cross in order to discuss the foundation of a society for action against poliomyelitis. After further deliberations with doctors and Social Welfare authorities the National Association Against Poliomyelitis was formally presented to the public in 1950 and given the tasks of (1) emergency preparations in view of anticipated poliomyelitis epidemics (2) convalescent treatment and rehabilitation of poliomyelitis victims (3) information and propaganda (4) scientific research (5) international co-operation.

From the proceeds of a fund raising campaign the Swedish Red Cross was able to increase the stocks of technical appliances for poliomyelitis patients in the so-called medical loan depots of which there are at present approximately 190 in the 24 Red Cross districts and to provide suitable material for diversional therapy. The local Red Cross districts established special services for assistance to and rehabilitation of poliomyelitis patients such as home visits transport assistance various loans from Red Cross depots diversional therapy club activities swimming courses etc. The National Association Against Poliomyelitis has undertaken to cover charges incurred by the Red

Cross Rehabilitation Centers. Members of Voluntary Aid Detachments and Red Cross auxiliary nurses have performed outstanding duties in connection with artificial respiration treatment and their work has been favorably acknowledged by the official medical authorities.

The Swedish Red Cross is always prepared for emergency measures in case of an outbreak of poliomyelitis and Red Cross volunteers are being used as assistants to doctors and nurses in the present vaccination campaigns for preschool children and members of the armed forces.

**Swiss Red Cross.** This society is willing to participate as far as possible in the fight against poliomyelitis and consequently is an active member of the Swiss Association for Poliomyelitis. Should an epidemic break out the Swiss Red Cross would make available its hospital services blood transfusion service (for supplies of gamma globulin) and voluntary personnel (auxiliary nurses who have married and given up their profession) to assist the overworked nursing staff.

Several local sections have established a service for the chronically ill. The service has a qualified physiotherapist and can co-operate in the rehabilitation of patients.

**Thai Red Cross.** Up to the present Thailand has been left untouched by poliomyelitis epidemics. However since 1954 the Thai Red Cross has admitted into its Chulalongkorn Hospital a total of 13 children suffering from infantile paralysis.

**French Red Cross.** Certain sections of this society have ambulances equipped with respiratory appliances for the transportation of poliomyelitis patients. The French Red Cross also organizes reserve teams specialized in the care of poliomyelitis victims.

**Italian Red Cross.** This society has an institute at Malcesine on Lake Garda. The center is equipped for anesthetic treatment and the surgical treatment of inveterate sequelae of poliomyelitis.

The institute also has the necessary material for diagnosis hydrotherapy (swimming pool baths etc.) massotherapy electrotherapy and the most modern installations for surgery. It can accommodate about 150 infantile paralysis victims and more than 100 beds are available for infantile orthopedic cases.

## THE ROLE OF THE LEAGUE OF RED CROSS SOCIETIES IN TWO RECENT POLIOMYELITIS EPIDEMICS

**Argentina** In February 1957 an epidemic of poliomyelitis broke out in Argentina. Some 2,300 cases were reported of which 10 per cent terminated fatally and on the average the victims were from 3 to 4 years old.

A telegram was sent immediately from the League Secretariat offering help to the Argentine Red Cross.

With the support of the Ministry for Foreign Affairs and the Association for Infantile Paralysis the Federal German Red Cross spontaneously sent a delegation of 3 doctors (2 pediatricians and 1 anesthetist) and 2 hospital nurses all of whom were Red Cross specialists in the care of poliomyelitis patients. The delegation took with them an iron lung, several respirators and certain medicaments.

The Italian Red Cross also sent 2 iron lungs.

The American Red Cross advised the National Foundation for Infantile Paralysis and contacted the United States Government. An expert was then sent to Buenos Aires in order to survey the situation and 21 iron lungs were dispatched by air.

The Czechoslovakian Red Cross sent a gift which was used for the purchase of a large motor conveyance for the National Institute for the Rehabilitation of the Handicapped in order to facilitate the transportation of patients to their treatment centers.

A gift of hospital linen and bandages was sent to Buenos Aires by the Indian Red Cross.

Meanwhile of course the Argentine Red Cross was taking a most active part in the fight against the disease. From the start of the epidemic this society had sent nearly 200 nurses to various hospitals and rehabilitation centers. Two iron lungs had been made available and the society made collections among the public for the purchase of 3 more. It also collected clothes, food, medicaments, books and toys.

Hungary Following the recent outbreak of poliomyelitis in Hungary the Hungarian Red Cross has asked the League if it could arrange for the loan of 20 iron lungs and other respiratory apparatus as quickly as possible.

In the early stages of the epidemic the delegate of the International Committee of the Red

Cross at Budapest transmitted to the Hungarian Red Cross a quantity of gamma globulin.

According to information given to the delegate of the International Red Cross at Budapest by the Hungarian Ministry of Public Health more than 750 cases of poliomyelitis have been registered. A number of these are children. 70 patients are seriously ill and a majority of these cases require respiratory apparatuses.

The League has launched an appeal to a number of national societies believed to be in a position to loan such equipment to the Hungarian Red Cross. Subsequently the British Red Cross Society has announced the dispatch of 2 iron lungs by air and the Federal German Red Cross is preparing to send 12. The Canadian Red Cross Society has offered to send if necessary gamma globulin supplies.

In addition the Swedish Red Cross has decided to send 2 poliomyelitis experts. Dr. Jonas Lindahl and Dr. Werneman to Budapest to survey the situation and determine needs. On arrival in the Hungarian capital they will confer immediately with the Hungarian Red Cross, the Ministry of Public Health and the delegates of the International Committee of the Red Cross and the League.

## AUSTRIA

### PROF. DR. KARL KUNDRATITZ

Oesterreich ist in den letzten Jahren von keiner schweren Polio-Epidemie heimgesucht worden. Die letzte grossere Epidemie war im Jahre 1947 mit 3508 Erkrankungen und einer Mortalität von 86%. Die Zahl der Erkrankungen sank dann z. B. im Jahre 1952 auf 198, um dann allmählich wieder anzusteigen. Voriges Jahr kam es wieder bis zu 1018 Erkrankungen mit 92 Todesfällen.

Seit 1954 ist es nun auch in Oesterreich möglich Epidemien virologisch zu untersuchen. So konnte bei 4 Epidemien nur Typ 1 gefunden werden. Bei sporadischen Fällen, besonders bei meningalen Formen, wurden die beiden anderen 2 Typen des Virus häufiger gefunden.

Bemerkenswert waren voriges Jahr zwei Anstaltsendemieen: eine in einer Säuglingsstation mit 8 Erkrankungen; bei diesen beiden Endemien konnte immer nur der Typ 1 nachgewiesen werden. Bei einer Endemie dieses Jahr in einem Kinderheim konnte bei ungefähr 500 Kindern die in einem direkten oder indirekten

Kontakt mit einigen Erkrankungsfallen standen ohne klinische Erscheinungen oder geringen Minimalsymptomen bei einigen Kindern in 80 / poliovirus wieder nur vom Typ 1 im Stühle gefunden werden

Es stehen in Wien bisher nur 2 Untersuchungsanstalten und zwar das hygienische Institut der Universität und die bundesstaatliche Untersuchungsanstalt zur Verfügung, um Gewebekultur Untersuchungen durchzuführen verbunden mit der Bestimmung neutralisierender sowie komplementbindender Antikörper

Von Bedeutung ist dass in Oesterreich jetzt oftmals andere Viruserkrankungen des Zentralnervensystems mit dem klinischen Bild einer Poliomyelitis ablaufen auch mit Lahmungen und Todesfällen Neben anderen schon bekannten Viren wurde im vergangenen Jahre auch ein neuer Mause pathogener Stamm isoliert

Grosses Interesse wenden die Gesundheitsbehörden der aktiven Schutzimpfung zu Es konnte aus Mangel an Impfstoff noch keine allgemeine Impfung durchgeführt werden Es wurden aber bereits im Jahre 1955 mehrere 1000 Kinder mit dem Impfstoff der deutschen Behringwerke geimpft und in diesem Jahre ebenfalls bei 1000 Kinder mit dem Impfstoff der amerikanischen Fa Pitman Moore

Nun werden bereits die organisatorischen Vorbereitungen getroffen um freiwillige Impfungen in grosserem Ausmasse durchzuführen da auch vom Nachbarland Ungarn eine Einschleppung zu befürchten ist

1954 hat in Oesterreich das Institut für Haemoderivate die Produktion von Poliomyelitisvakzine begonnen (nach Salk) und diese dürfte uns auch bald zur Verfügung stehen

Für die verschiedenen Fragen die mit dem Gesamtproblem der Polio zusammenhängen haben die Gesundheitsbehörden in Obersten Sanitätsrat die massgebende beratende Körperschaft Für Behandlungszentren mit den notwendigen Beatmungsapparaten ist vorgesorgt Für den Transport stehen Rettungsautos vom Roten Kreuz mit Beatmungsapparaten zur Verfügung

## BELGIAN CONGO

Dr J P DELVILLE

Le fait saillant de la poliomyélite au Congo Belge est que cette maladie ne touche les congolais que dans le tout jeune âge et ce avec

relativement peu de dégâts alors que les populations blanches lui paient un bien plus lourd tribut et sont touchées à tout âge

Depuis 1945 le nombre de cas de poliomyélite est en progression constante tant chez les congolais que chez les européens avec cependant une incidence beaucoup plus élevée chez ces derniers

Pendant l'année 1954 année d'épidémie de poliomyélite pour laquelle nous disposons de données suffisamment précises l'incidence de cette maladie fut de 4 pour 10 000 chez les européens contre seulement 0.64 pour 10 000 chez les congolais

Cette épidémie sevit plus particulièrement à Elisabethville fin 1954 début 1955 et nous avons pu l'étudier de près

Tous les cas sont survenus entre novembre 1954 et mars 1955 soit pendant la saison des pluies qui fut particulièrement pluvieuse Il est à noter qu'à Elisabethville les cas de poliomyélite surviennent toujours presque exclusivement pendant la saison des pluies

Pendant cette épidémie l'incidence fut de 2.08 / 0 chez les européens contre 0.43 / 0 chez les congolais avec une mortalité respectivement de 12.5 / et de 7.5 / des cas La mortalité fut donc également nettement supérieure chez les européens

La répartition des cas en fonction de l'âge reprise dans le tableau ci-dessous mérite également d'être signalée

	CONGOLAIS	EUROPÉENS
De 0 à 2 ans	87%	25.0%
Moins de 10 ans	100%	67.5%
Plus de 10 ans	0%	37.5%
Plus de 20 ans	—	33.0%

Pour une autre épidémie survenue à Bukavre en 1956 chez les congolais 88 / des cas avaient moins de 2 ans et 41 / moins de 1 an et aucun cas ne fut constaté au-delà de l'âge de 6 ans

Nous avons également pu faire l'étude virologique de ces deux épidémies et il est intéressant de noter que les 18 souches de virus poliomyélitique de l'épidémie d'Elisabethville et les 15 souches de l'épidémie de Bukavre que nous avons pu isoler sur cellules Hela furent toutes du type 1

De ces deux épidémies nous pûmes en outre

isoler 28 souches de virus Cotsackie dont 14 en association avec le virus poliomyelitique

Toutes les souches provenant de l'épidémie d'Elisabethville furent du groupe histologique A alors que l'épidémie de Bukavu nous fournit des types A et II

Signalons que nous avons également pu après isolement du virus Cotsackie II à partir du liquide céphalo-rachidien chez deux cas de méningite aseptique

Alors que les cas de poliomyélites paralytiques ne nous ont permis que l'isolement de souches de type I l'étude des anticorps sériques montre que les autres types sont tout aussi répandus

Les résultats de l'étude des anticorps sériques chez les congolais sont en parfaite concordance avec les constatations épidémiologiques

Les anticorps hérités de la mère disparaissent pendant la 1<sup>re</sup> année de la vie. Mais déjà à partir de l'âge de 6 mois nous voyons apparaître des anticorps d'immunisation active et vers l'âge de 6 ans pratiquement 100 % des congolais possèdent des anticorps vis-à-vis des 3 types de virus poliomyelitique

La vaccination antipoliomyelitique a été entreprise début 1957 dans les écoles européennes des grands centres mais sans que celle-ci soit rendue obligatoire

## BELGIUM

DR. PIERRE RECHT

### SITUATION EPIDEMIOLOGIQUE DE LA POLIOMYELITIS EN BELGIQUE DEPUIS 1914

Après l'année 1954 au cours de laquelle il n'y eut que 198 cas de poliomyélite la Belgique a connu en 1955 et 1956 deux épidémies successives qui ont fait en 1955 979 victimes et en 1956 1038 victimes

Le total des cas de poliomyélite constatés dans notre pays depuis 1914 soit pendant 17 ans est de 5389 ce qui nous fait une moyenne de 317 cas par an pour une population de  $\pm 8700000$  habitants soit 37 cas par 100000 habitants

L'épidémie de 1955 de 979 cas au cours de laquelle 34 décès ont été constatés représente une moyenne de 11 par 100000 habitants. L'épidémie de 1956 de 1038 cas avec 30 décès représente une moyenne de 12 cas par 100000 habitants

Les variations saisonnières de la maladie

indiquent que les épidémies de 1945 et 1957 se sont surtout déroulées de juin à octobre celle de 1955 de juillet à décembre et celle de 1956 de juillet à novembre. La maladie garde dans l'ensemble un caractère de prédominance estivo-automnale, le mois de février connaissant le minimum de cas

L'étude des groupes d'âges touchés par la poliomyélite indique que la période de prédilection se situe entre 0 et 10 ans

	ENFANTS DE 0 A 10 ANS	ADULTES DE PLUS DE 20 ANS
En 1952	68% du total des cas	13%
En 1955	80% du total des cas	11%
En 1956	70% du total des cas	15%

La poliomyélite reste surtout une infection infantile mais au cours de ces dernières années on a observé une augmentation du nombre des cas qui touchent les adultes la gravité des formes augmente avec l'âge et notamment les formes respiratoires sont de loin plus fréquentes au-delà de 20 ans

Quant à la distinction entre les formes paralytiques et non paralytiques les statistiques de notre pays indiquent qu'en 1952 80 % des cas signalés étaient des formes paralytiques en 1955 78 % en 1956 63 %

Le pourcentage plus faible des formes paralytiques au cours de l'année 1956 est du vraisemblablement à deux raisons d'une part une déclaration plus fidèle des cas de poliomyélite même nonparalytique et d'autre part l'incidence au cours de l'année 1956 de nombreuses atteintes méningées dont certaines ont été sans certitude rattachées à la poliomyélite

En conclusion la poliomyélite dans notre pays est une maladie à prédominance infantile dont le taux d'endémicité se situe pour les deux dernières années entre celui de la diphtérie et de la scarlatine et qui nous permet de classer notre pays parmi les nations favorisées. Par rapport aux Etats Unis et aux Etats Scandinaves la fréquence des cas est actuellement de 3 à 4 fois moindre même en période d'épidémie

### EPIDEMIE DE 1956 ET MENINGITE EPIDEMIQUE

De juin à décembre 1956 de nombreux pays de l'Europe Occidentale ont été touchés par une épidémie de méningite aseptique

En Belgique l'agent étiologique de 254 cas a été identifié comme un virus ECHO appartenant au type 9\*

Des échantillons de selles reçus de Suisse contenaient aussi du virus ECHO du type 9 et la source isolée au Pays Bas a été trouvée semblable à celle trouvée en Belgique.

Ce virus était aussi aisément découvert et mis en évidence à partir du liquide céphalo rachidien qu'à partir des selles.

Les produits de gorge contiennent le virus mais uniquement quand il est recueilli au tout premier jour de la maladie.

Certains virus ECHO belge type 9 se sont révélés être pathogènes pour le souriceau quand ils sont inoculés après passage sur cultures de tissu. L'inoculation directe du virus à partir d'échantillons de selles n'a donné aucun symptôme. Aucune de ces souches n'a été neutralisée par des sérums de types connus.

La méningite aseptique que nous avons connue en Belgique était caractérisée par des symptômes méningés et était parfois accompagnée d'exanthèmes. La pleocytose était toujours marquée plus de 5000 éléments par mm. La maladie possédait un haut degré de contagiosité. De nombreux exemples ont montré que tous les membres d'une famille, enfants et adultes, peuvent être atteints.

## ORGANISATION DE LA LUTTE CONTRE LA POLIOMYELITIS

Nous avons organisé en 1954 à l'initiative du Ministère de la Santé Publique et de la Famille un réseau respiratoire belge destiné à traiter les complications respiratoires de la poliomyélite. Huit centres dont les 4 centres universitaires ont été spécialement équipés en appareils à respiration artificielle. Un service d'ambulances spécialement affectées au transport des malades atteints de poliomyélite a été organisé avec un seul numéro d'appel téléphonique pour toute la Belgique. En raison de l'automatisme presque complète du réseau téléphonique il est possible pour un médecin d'appeler de n'importe quelle commune de Belgique le service central qui se charge dès lors de prévenir le secteur de transport le plus proche du domicile du malade. Ce service a fonctionné au cours des épidémies de 1955 et 1956 et a effectué plus de 500 transports.

Chaque secteur d'ambulance a un rayon de  $\pm 25$  km. Les centres de traitement sont répartis de telle manière que sauf pour le Sud du pays qui heureusement est peu peuplé la durée maximum du trajet en ambulance est de 1 heure.

Nous sommes persuadés que cette initiative a permis de sauver de nombreuses vies humaines et que la mortalité de la poliomyélite et la gravité des atteintes en ont été améliorées.

L'Etat est intervenu très largement dans l'achat de l'équipement des centres destinés à recevoir des malades atteints de troubles respiratoires. Il est en effet difficile de faire supporter par un organisme d'assistance locale les frais d'achat de poumons d'acier ou d'appareils respiratoires à pression positive coûteux alors que ces centres sont destinés à recevoir des malades venant d'autres régions. Aussi est-ce le Gouvernement qui a fait l'acquisition des appareils et les a mis à la disposition des centres agréés.

Des échanges de matériel et de personnel ont eu lieu en 1956 lors de l'épidémie qui a provoqué vers les mois d'octobre et de novembre un nombre très élevé de formes respiratoires.

La formule que nous avons adoptée pour installer les centres de poliomyélite aigüe est inspirée des recommandations parues après le 7<sup>e</sup> symposium européen de la poliomyélite qui s'est tenu à Paris en 1954.

Nous pensons qu'un centre destiné à recevoir les malades aigus doit être situé dans l'enceinte d'un hôpital général et peut ainsi bénéficier de l'intervention des différentes spécialités médicales intervenant dans la lutte contre les complications respiratoires.

Il existe néanmoins en Belgique un centre privé qui est spécialisé dans la poliomyélite et qui traite les formes aiguës et chroniques.

Mais 6 centres sont installés dans les services de contagieux et 1 dans un service de neurologie situés dans des hôpitaux généraux.

L'équipement respiratoire actuel de la poliomyélite comprend une quarantaine de poumons d'acier, une douzaine d'appareils Engstrom, une vingtaine d'autres appareils à pression positive et une dizaine de cuirasses.

## LA VACCINATION

Devant l'importance du problème de l'application de la vaccination antipoliomyélique le Ministère de la Santé Publique et de la Famille a constitué en avril 1955 une Commission mixte

\* ECHO 1  
T.P. A) a  
125 44 195  
Type 9 (n.w.m.b.e. of Cov. a k group  
Juse f p d m n n s t c c n

s'adressent à des étudiants de l'Université du même âge 23 % seulement des sujets ont des anticorps contre les trois types

Un second exemple montre l'influence des conditions économiques et sociales les enfants que ont fait l'objet des examens préliminaires aux vaccinations appartiennent à des milieux d'ingénieurs de professeurs et surtout de médecins Il est remarquable de constater que ces enfants se révèlent très mal immunisés contre la poliomyélite et que même à 14 ans 11 % seulement possèdent des anticorps contre les trois types

La Figure 2 demande les explications suivantes Trois courbes sont portées sur le même graphique La première (traits pleins) a été dessinée d'après les renseignements fournis par le Dr Recht au sujet des 1 078 cas de poliomyélite survenus en 1956 elle donne selon l'âge le pourcentage cumulé des cas de poliomyélite sur la totalité des cas enregistrés 45 % avaient 4 ans ou moins 72 % avaient 9 ans ou moins 87 % avaient 19 ans ou moins

De la même façon a été établi le pourcentage cumulé des sujets exempts d'anticorps (traits

) et des sujets non complètement immunisés (traits )

On constate que les cas de poliomyélite se répartissent suivant la courbe des sujets exempts d'anticorps plutôt que suivant celle des sujets porteurs d'une immunité nulle ou partielle Ceci indique que les risques de contracter une poliomyélite cliniquement apparente sont surtout grands lors de la première rencontre avec le virus plutôt que lors des deux suivantes et que par conséquent la prédisposition individuelle semble jouer plus que les facteurs occasionnels Lorsque le virus poliomyélique fit son apparition en Belgique lors de l'épidémie de 1956 il se trouva en présence d'une population où les sujets exempts d'anticorps se retrouvaient à raison de 43 % dans le groupe d'âge de 4 ans et moins or les ravages que la maladie causa furent précisément constatés à raison de 45 % chez ces mêmes enfants de 4 ans et moins

Dans les groupes d'âges plus élevés les courbes divergent entre 5 et 9 ans il y a 19 % de sujets sans anticorps mais 27 % de cas de poliomyélite Si cette divergence est significative elle est un indice que chez les sujets exempts

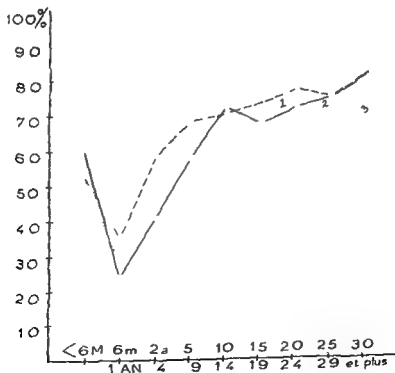


FIG 3 (See text)

d'anticorps les risques de contracter une poliomyélite clinique augmentent avec l'âge. Or cette divergence doit être significative car ces courbes donnent des pourcentages cumulés et convergent donc toutes normalement vers le point 100 /

Pour qu'une divergence se dessine il faut que l'un des facteurs se modifie avec l'âge ce facteur serait sans doute la réceptivité individuelle aux complications nerveuses poliomyélitiques. Le même écartement entre les deux courbes se retrouve encore si ce sont les cas de poliomyélite de 1952 que l'on porte sur les graphiques au lieu de ceux de 1956.

Il procède à un autre calcul Olin en Suède a démontré également que la même rencontre avec un virus poliomyélitique comporte d'autant plus de dangers que le sujet est plus âgé.

Figure 3 montre la comparaison entre le pourcentage des individus immunisés contre le type 1 au moins et celui des sujets immunisés contre les types 2 et 3 au moins. L'immunité

contre le type 1 est un peu plus précoce que pour les deux autres types. En fait on devrait s'attendre à une différence beaucoup plus forte car depuis trois ans que des recherches de virus polio sont effectuées en Belgique sur une assez grande échelle chez des malades paralytiques et chez des porteurs sains les souches de type 1 se retrouvent toujours dans 80 à 90 % de cas contre 5 à 10 % quand il s'agit du type 2 et du type 3.

Or la même discordance se retrouve dans beaucoup de pays il y a une prédominance très élevée de la souche 1 et une prédominance peu marquée de l'immunité contre le type 1. Ces deux faits ne peuvent être conciliés qu'en supposant qu'il circule parmi la population des virus de types 2 et 3 que nous détectons par les anticorps qu'ils font apparaître mais que nous ne pouvons mettre en évidence directement au laboratoire peut-être parce que ces souches comme la fait remarquer M<sup>me</sup> Quersin ne sont pas capables de causer des lésions au niveau des cultures de tissus employées habituellement.

Olin G. et W. In T. A. h. Nr. 1 sh. 7 191  
1957

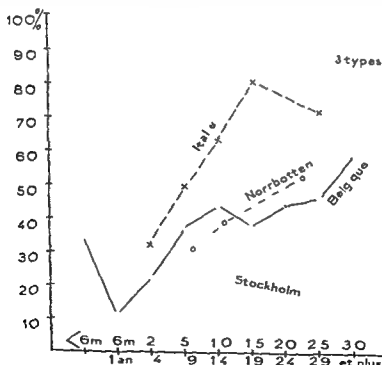


FIG. 4 (See text)



(souches immunisantes mais non cytopathogènes)

Figure 4 montre les anticorps contre les 3 types de virus. Comparaison de l'état d'immunité de la population belge à celui de la Lombardie Italie et à celui de deux régions suédoises dont l'une (Stockholm) connaît généralement un nombre de cas beaucoup plus élevé que l'autre (Comté de Norrbotten).<sup>1</sup> On voit que l'immunité belge vaut à peu près celle de la région suédoise la moins atteinte par la maladie.

Figure 5 montre les anticorps contre le type 2 au moins encore une comparaison entre l'état d'immunité de différents pays.

## BRAZIL

DR OSWALDO P. CAMPOS

We have presented to the past conferences statistics of infantile paralysis cases admitted to the Jesus Hospital in Rio de Janeiro. In Brazil it has been our purpose to demonstrate that the disease constitutes a twofold problem. First

<sup>1</sup> See footnote on p. 13.  
<sup>2</sup> Gioanardi, A. Monaci, V. et Bonetti, F. *Ille Symposium* 1956, pp. 47-54.

poliomyelitis is a very serious health problem which has increased in importance each year and second it strikes in over 93 per cent of cases the 0-5 year age group thus being obviously an extremely difficult orthopedic problem.

During the last 3 year period from June 1954 to May 1957 1 058 new cases of poliomyelitis were admitted to the Jesus Hospital which with the addition of 136 private cases brings the total to 1 194 which is the number of new victims of infantile paralysis brought to us for our personal observation.

Table 1 shows that the pattern of the disease has not changed the 0-3 year age group being more prevalent with a percentage of 88.10.

Of the 1 194 cases 146 were stricken before the age of 6 months reflecting a percentage of

TABLE 1 AGE INCIDENCE

AGE IN YEARS		INCIDENCE (PERCENTAGE)
0-1	702 cases	58.79
1-3	350 cases	29.31
3-5	68 cases	5.69
5-10	33 cases	2.77
10 up	18 cases	1.50

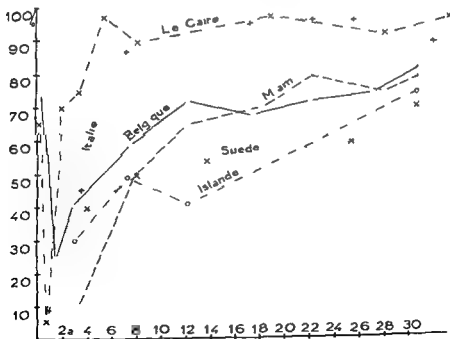


FIG 5 (See text)

12.22 which is a much higher rate than the 3.76 per cent reported by us in 1954.<sup>3</sup>

These data only confirm what we have stressed previously that poliomyelitis infection due to very poor hygienic conditions still prevailing in both large and small cities throughout Brazil is widespread.

The infantile type is highly prevalent. Dr Mauricio Martins da Silva and Dr Jerome T. Syverton<sup>4</sup> working along the same line as other investigators in many parts of the world recently have fully confirmed these clinical and epidemiologic assumptions. Blood samples of 111 children living in Rio de Janeiro were sent to the University of Minnesota for serologic typing and specific polio antibody titer determinations. The 111 children submitted to this investigation were selected from 3 different socioeconomic levels.

Group A consisted of 29 children taken from the slums or nearby densely populated rural areas where living conditions are very primitive and promiscuity a predominant characteristic.

Group B consisted of 58 children taken from middle-class families living in small houses or apartments having running water and other hygienic facilities.

Group C consisted of 24 children taken from the upper wealthier class. It is noteworthy that according to the epidemiologic viewpoint there is not a complete separation of the slums in Rio de Janeiro. The slums and the wealthy sections are both rather mixed in a very indiscriminate manner.

The search for the presence and the titer of specific antibodies against polio viruses as determined by tissue cultures showed Group A to have antibodies against the 3 types of polio viruses in 79 per cent of the cases. Group B in 53 per cent of the cases while Group C in only 29 per cent.

The presence of antibodies against at least 1 of the 3 types of viruses was found in 100 per cent of Group A, in 97 per cent of Group B and in 96 per cent of Group C, the average age being 8, 11 and 18 years respectively. Thus it proves that latent immunity is acquired more rapidly in the poorer classes than in the groups better endowed economically. Although no definite data are available the attack rates seem to diminish with increasing age among the poorer classes. The classes forming Groups B

and C of the Martins da Silva and Syverton investigations seem to be more susceptible to the disease although there is not any noticeable difference in age selection patterns among the 3 different groups. We would like to call attention to the observations referred to in our report to the Second International Poliomyelitis Conference<sup>5</sup> showing that paralytic polio is a rarity among underprivileged children living in the slums of Rio which constitute the majority of Group A of the above mentioned authors. During epidemic times however because of changes in virus virulence or in host conditions we have seen children with nutrition deficiency stricken by polio which otherwise seems to be a very uncommon occurrence.

After the 1953-54 epidemic polio is certainly undergoing a transformation in the Federal District and the surrounding cities in the State of Rio de Janeiro it being definitely on the upward trend and recent observations warrant the assertion that the same is true for many regions of the country.

The admission rate of acute cases to the Jesus Hospital prior to the 1953-54 epidemic was 3 to 4 cases each month showing a flare up during the hot and damp summer season. After the 1953-54 epidemic which was reviewed in our report to the Third International Poliomyelitis Conference<sup>3</sup> the monthly rate of admission in the Jesus Hospital stayed at the much higher level of an average of 27 cases as one can see in Table 2 as for example for the year of 1956.

In 1956 there were 211 cases with sequelae admitted to our hospital. However during that year many of those stricken came to the hospital only after the acute stage was over. This brings the attack rate well over 10 per 100,000 of popu-

TABLE 2. ADMISSION RATE OF ACUTE CASES TO JESUS HOSPITAL

January	8
February	11
March	12
April	20
May	16
June	21
July	32
August	47
September	39
October	33
November	22
December	14

lation and one can draw the conclusion that after the 1953-54 epidemic infantile paralysis reached an almost permanent epidemic level in the Federal District. It will be worthwhile to note that the 1953-54 epidemic of Rio de Janeiro with an incidence of 21.88 in each 100,000 of population according to Aristides Paes de Almeida<sup>1</sup> and J. G. Lacorte,<sup>2</sup> reached a higher level than the recent Argentine epidemic which showed the figure of 17.5 in each 100,000 inhabitants.

We would also like to mention the fact that the clinical manifestations of the disease have not changed much after that terrible epidemic, the spinobulbar and the encephalitic types still occurring with great frequency.

### VACCINATION PROGRAM

In the latter part of 1956 with the mounting evidence as to the safety and the effectiveness of the Salk poliomyelitis vaccine and taking the opportunity of the export release of the vaccine by the American Government a pioneering program of immunization was planned for the city of Rio de Janeiro.

In Brazil as in many parts of the world a lack of enthusiasm for the Salk vaccine still exists and some resistance to its use by parents and even doctors is encountered. This of course applies in any type of preventive vaccination which seldom is freely accepted by the general public. The decision to test the Salk vaccine in Rio de Janeiro was taken after Dr. Raphael de Souza Parva, Director of the Department of Pediatrics in the Federal District and his assistants Drs. Alvaro Aguiar and Otavio da Veiga returned from the 8th International Conference of Pediatrics held in Copenhagen in 1956. They were very much impressed by the papers presented on the subject during that conference. We did stress to Dr. Souza Parva the necessity of such a trial on our return from the United States in April, 1956.

The different aspects of the problem were analyzed and the nature of poliomyelitis in Brazil was particularly taken into consideration on account of questions raised by some of our eminent specialists who based their objections on recommendations made in Stockholm by the World Health Organization in January 1956.<sup>3</sup> Finally an elaborated program was presented to Dr. Darcy Monteiro, Health Commissioner of

the Federal District. This met with his enthusiastic approval and in November 1956 a vaccination trial was begun in Rio de Janeiro in a region where polio is very common, endemic and essentially infantile.

The first lot of vaccine was imported by the Municipal Government and because of legal technicalities was allotted only to the 16 different pediatric districts into which the metropolitan and urban areas of Rio de Janeiro are divided. In these health centers any child within the 6 months to 3 year age group the group most commonly stricken could be vaccinated free of charge. A special committee to work under Dr. Souza Parva's direct supervision was appointed. This committee issued strict norms and regulations for the use of the vaccine and a well-organized system of control cards for necessary references and statistics was established. A special identification card is given to the family as a memento not only for the Salk vaccine but also for recording a complete polio vaccination program as well as any other vaccines given to the child up to the age of 5 years. This card in a simple manner gives educational information about the Salk vaccine, the idea being to secure the confidence of the parents and to free them from undue apprehensions.

The results of the first 3 months of the vaccination program in spite of the favorable publicity fell very short of the expectations. From November 13, 1956, to February 2, 1957, only 14,053 children were inoculated and what was very discouraging only 1,650 returned for the second inoculation which was almost an anticipation of a complete failure of our program.

In February 1956 the situation was reevaluated and it was decided that this age group should be extended up to 6 years. At this time an intensive campaign was directed to the general public through the press, the radio and television which imparted information on the Salk vaccine, stressing the necessity of the parents co-operating with the health authorities and warning the public against the possible rise of the incidence of polio in the months of March, April and May. This drive was very gratifying, raising the presentation for vaccination considerably, and by June 8 the number of first inoculations had reached 51,334.

## Reports of Official Delegates

Unfortunately the attendance for the second inoculation again fell far below a desirable figure since only 27,535 children showed up for this indispensable dose which is the insurance of a good protection against paralytic polio.

We are now calling on private organizations, other health services, military hospitals etc to co-operate in this vaccination program so that most of our infantile population which is estimated to be of about 350,000 from 0 to 6 years can derive the benefits of the only weapon for fighting paralytic poliomyelitis at our disposal at the present time. We realize that compulsory vaccination against polio for obvious reasons lies many years ahead.

This pioneering program is also educational in scope. It serves mainly to ward off the fear created about the poliomyelitis vaccine because of the few accidents which occurred during 1955. It is well known that they were discovered promptly and corrected to the point where it can be said that there is no other biologic product which is surrounded by as many safe guards and tests of safety as the Salk vaccine which is being distributed at the present time by the various manufacturers. We are very happy to say that this goal has been unmistakably attained which is proved by the fact that the Department of Health and Welfare (Secretaria Geral de Saude e Assistencia) of Rio de Janeiro is now receiving daily from all over Brazil requests for advice about the use of the vaccine. I am sure that from now on no responsible practicing physician will assume the unforgivable responsibility of contraindicating the polio vaccine only to see his patient stricken a little later by this terrible disease as has happened in 3 tragic instances brought to my knowledge.

Until now we have registered only 3 cases of paralytic polio after the vaccination and their histories follow in detail without unnecessary comment.

Case 1 NAI, female 1 year 3 months of age. First dose of Salk vaccine was administered on January 4, 1957. On January 12 temperatures of 103 and 104 were recorded. She was restless and shaking of the head persisted for 72 hours followed by complete recovery. On January 23 she fell sick again. A temperature of 102 was reported and the patient complained that her left leg was extremely sensitive to touch.

This picture lasted for about 3 days. Later the patient was unable to walk because the left leg was flail. Examination on March 13 revealed slight atrophy of the left thigh and leg.

Muscular examination showed the following:

	Weak
Gluteus maximus	Trace
Biceps	0
Tibialis Anticus	0
Tibialis posterior	0
Reflexes were as follows	0
Left patellar	0
Left achilles	

Case 2 HJS, male 11 months old. First dose of Salk vaccine was given on December 7, 1956. The second dose on January 7, 1957. On March 31 the patient was very sick and vomiting with high temperature. Constipation and urine retention. On the third day there was flaccid paralysis of both lower limbs followed by paralysis of the upper extremities and marked involvement of the respiratory muscles particularly the intercostal group. Spinal fluid cells 86, proteins 0.15 per cent, sodium chloride 6.6 gms per cent, glucose 0.48 per cent. The child recovered from the acute stage but there was extensive residual paralysis.

Case 3 LPR, male 5 years old. First dose of Salk vaccine was given on February 13, 1957. The second on March 13, 1957.

On April 16 the boy had a high temperature without any other particular symptoms. A few days later he began to limp and walked with the left foot turned inward. Examination revealed slight atrophy of the left leg. He walks with the left foot slightly dropped and inverted. The peroneal and the tibial anticus are very weak. The left peroneal reflex is absent.

Fortunately the General Department of Health and Welfare in the Federal District is determined to carry on with this poliomyelitis vaccination program its extent being limited only by the present export restrictions of the United States. It is also the desire of the Committee of this program to co-operate with the National Foundation for Infantile Paralysis in any investigations as to the effects of the vaccine in regions where poliomyelitis still strikes under its primitive aspects and where the children are constantly exposed to the virus can develop a permanent natural immunity under the protection against paralytic polio by the vaccine.

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TABLE 3 NUMBER OF CLINICAL CASES IN THE STATE CAPITALS OF BRAZIL\*

CAPITALS	YEARS											
	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955
Manaus	—	—	—	1	—	—	—	—	1	—	—	3
Belém	—	—	—	1	1	5	1	6	—	3	—	2
Sao Luiz	—	—	—	—	—	3	—	3	—	—	—	1
Teresina	—	—	—	—	—	—	—	—	—	—	—	—
Fortaleza	6	1	1	1	12	9	—	9	2	3	3	1
Natal	—	—	—	—	1	—	—	1	—	1	—	—
João Pessoa	4	1	—	—	—	—	—	1	1	—	11	—
Recife	3	3	29	2	2	5	1	6	4	3	1	6
Maceió	2	—	4	1	—	9	1	—	3	—	1	1
Aracaju	—	—	1	—	1	—	1	—	1	—	—	—
Salvador	1	3	—	11	2	5	1	4	17	—	—	3
Vitória	—	3	1	4	1	2	—	—	5	—	1	1
Niterói	—	—	—	—	—	5	1	2	2	56	13	—
Rio de Janeiro	64	45	46	41	40	22	25	24	37	746	357	124
Sao Paulo	17	27	10	29	11	4	3	14	29	136	42	127
Curitiba	—	1	—	2	3	2	—	2	5	10	—	16
Florianópolis	1	11	7	3	3	5	5	14	—	3	1	—
Porto Alegre	7	10	68	5	27	68	15	12	15	19	18	—
Belo Horizonte	2	—	39	3	3	2	2	21	22	20	12	11
Goiânia	—	—	—	—	—	1	—	2	2	3	3	2
Cuiabá	—	18	1	—	—	—	—	—	1	—	—	—

\* Data from the *Boletim de Doenças* of the Health Ministry.

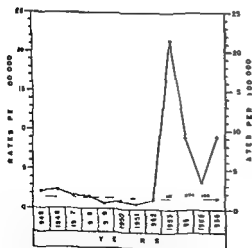


FIG. 7 Annual rates per 100 000 population of notified cases (residents) in Rio de Janeiro

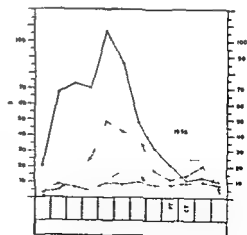


FIG. 8 Monthly distribution of cases (residents) in Rio de Janeiro.

demology of Poliomyelitis in Brazil (Speaker A Paz de Almeida) (2) Evaluation of the Immunity Against Poliomyelitis (Speaker J Travassos) (3) Vaccine with Virus (Speaker Prof Paulo de Goes) (4) Active Immunity Against Polio and Salk Vaccine (Speaker J Madureira Para) (5) Universal results of Anti poliomyelitis Vaccination (Speaker Prof J Martinho da Rocha)

The main conclusions reached were the following

1 Polio is a disease that occurs eventually or endemically in this country there are occasional epidemic outbreaks of minor or major extension

2 It is necessary to apply for an improvement of vital statistics on the epidemiology in order to afford better knowledge of the disease in our country

3 It is necessary to perform surveys concerning the epidemiology based on the serology of specific antibodies for poliomyelitis carried out with samples representative of the population concerning age and socioeconomic conditions

4 It is necessary to equip the laboratories in order to enable them to identify the types of viruses isolated in the epidemic areas

5 The available data show a variation in the specific age incidence with an overwhelming concentration of cases (paralysis) at low ages (80 per cent under 3 and 90 per cent under 5 years) as has already been recorded in certain areas

6 The frequency of extensive epidemic outbreaks recorded in certain areas (Rio de Janeiro, Sao Paulo, Niteroi and even smaller towns in the interior of some of the states) leads to the

assumption that there is a possibility that we are on the way to an epidemization of polio in those areas

7 It is virtually impossible to immunize with the Salk vaccine all Brazilian children even if one restricted the age to 5. Importing the vaccine would be too expensive there is a lack of trained personnel to give inoculations 3 times a year and the classifying of the types of viruses has not yet been done nor serologic studies made of the susceptibility

Due to the tremendous repercussion in our press of the Argentine polio outbreak in 1956 the Secretary of Health of the Municipality of Rio de Janeiro has started vaccination of children and so far there have not been any accidents in that city. Dr O Pinheiro Campos, Brazilian delegate will discuss this subject

The most objective and accurate data about immunization of children in Rio was published in the *Public Health Reports* Vol 71 April 1956 based on the work of Drs M Martins da Silva and J T Syverton done at the University of Minnesota. Their research gave the percentage of antibodies in 111 sera of children in Rio de Janeiro selected from 3 social groups. Group A consisted of 29 children (average age 8) living in poor conditions and in unhygienic quarters with lack of drainage and running water or in collective quarters. Group B was composed of 58 children (average age 11) from middle-class families living in modest houses or hygienic flats with running water and drainage with no crowding and with better economic standing. Group C consisted of 24 children (average age 18) of the upper class.

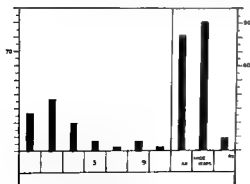


FIG 9 Percentage distribution of cases by age (residents) in Rio de Janeiro

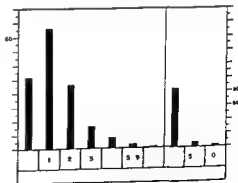


FIG 10 Specific case rates by age (residents) in Rio de Janeiro

TABLE 4 PERCENTAGE OF ANTIBODIES IN GROUPS A B C AND D AND E

GROUPS	NUMBER	AVERAGE AGE	PERCENTAGE WITH ANTIBODIES AGAINST			
			NO TYPE	1 TYPE	2 TYPES	3 TYPES
A	29	8.6	0	8.4	17.2	79.4
B	58	11.8	3.4	20.6	22.4	53.4
C	24	18.3	4.2	29.1	37.5	29.1
D	26	6.5	30.7	23.1	30.7	15.4
E	43	20-30	20.9	41.8	20.9	16.2

Table 4 shows the percentage of antibodies for the 3 groups as compared with Groups D and E of Minnesota. USA composed of sons of professors at the University or students. The percentages were 100 per cent in Group A, 97 per cent Group B and 98 per cent in Group C.

This work tends to demonstrate that a greater part of Rio's children, especially in the low socioeconomic classes, are naturally immune to polio.

### CANADA

DR F P NAGLER

The Canadian program of vaccination against poliomyelitis commenced in April 1955 as a co-operative project between the provincial departments of Health and the Department of National Health and Welfare. The several departments equally sharing the costs. The vaccine was produced by the Connaught Laboratories, Toronto, employing the Maudland technique and using the Mahoney, the MEFl and the Saukett strains in the preparation of the trivalent vaccine. Safety and potency tests, similar to those in use in the USA, were carried out simultaneously by the manufacturers of the vaccine and by the Laboratory of Hygiene of the Department of National Health and Welfare in Ottawa. Up to the present time about 60 lots of vaccine, varying from 300 to 600 liters each, have been submitted to the Department for testing. Most of these lots and a limited number of lots submitted by American manufacturers have been released by the Department for general use.

In 1955 about 800 000 Canadian children, mostly between the ages of 5 and 9 years, were inoculated with 2 to 3 doses, while up to the end of March 1957 another 7 000 000 children of preschool and school age received at least 2

injections of the vaccine. It is hoped that by the end of March 1958 all Canadians up to the age of 19 years (about 6 000 000) will have been vaccinated.

Before evaluating the results of the Canadian vaccination program of 1955 and 1956 it is necessary to consider the incidence rate of poliomyelitis in these years in relation to the rate of previous years. The rate of paralytic poliomyelitis in 1955 was 28 per cent of the average incidence rate for the 5 years (1950-1954) preceding while the rate in 1956 was 19 per cent of that average (1 900 cases/year, 1950-1954). The low incidence rate of the last 2 years has made evaluation of the results of the vaccination program somewhat difficult since the numbers of reported cases in both the vaccinated and the unvaccinated groups were relatively small. In 1955 for instance only 5 cases of paralytic polio were reported in 600 000 vaccinated children under observation and 51 cases in 890 000 unvaccinated children of the age group of 5-9 years. In 1956 11 paralytic polio cases in a total of 1 860 000 vaccinated children and 136 cases in 2 140 000 unvaccinated children of similar age groups were reported in Canada.

Considering the decline in the overall incidence rate of paralytic poliomyelitis in Canada during the past years it could be assumed that without vaccination this decrease would have occurred equally in all age groups. However, when calculating the expected incidence rate for 1956 it was noted that more cases than might have been expected occurred in the age group from 0-4 years, while the calculated and the observed rates of the age groups of 20 years and over were comparable. A consistently lower rate in observed cases as compared with the number of expected cases was found in the age



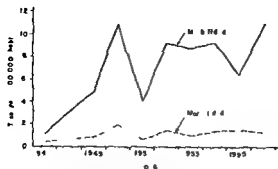


FIG. 11 Morbilidad y mortalidad por poliomyelitis en Chile 1947-1956

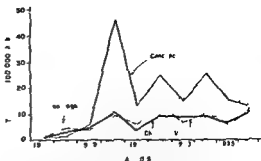


FIG. 12 Morbilidad por poliomyelitis en Valparaíso Santiago Concepción y Chile 1947-1956

groups between 5 and 19 years. Therefore it seems reasonable to assume that there has been a selective reduction of paralytic cases particularly in the school age groups since 1955. This reduction might well be a reflection of the protective effect of the Salk vaccine administered during the past 2 years. It is proposed to follow changes in the age distribution pattern of paralytic poliomyelitis as they may occur in Canada during the forthcoming years.

### CHILE

DR. HERNAN ROMERO

Las comunicaciones presentadas a las tres Conferencias anteriores (Nueva York 1948, Copenhagen 1951, Roma 1954) nos han dado oportunidad para señalar el aumento progresivo de la poliomyelitis en Chile que no ha hecho sino acelerarse ultimamente. En efecto la tasa de morbilidad se ha multiplicado más de 50 veces en este cuarto de siglo (0.2  $\times$  100 000 en 1933 y 11 en 1956). Los empujes de verano tienen clara tendencia ascendente y en ellos se pueden distinguir cinco epidemias sobresalientes en 1943-44, 1949-50, 1951-52, 1953-54 y 1955-56. Ordinariamente este prevalecer estacional no se ve claro en las tablas anuales porque nuestro año calendario comienza y termina en estío. Las mayores incidencias se dan entre Octubre o Noviembre y Abril a Mayo con la cumbre en Diciembre y Enero. Hasta hace poco las epidemias habían quedado circunscritas a las ciudades mayores. Recientemente se las ha observado también en poblaciones de mediano tamaño y aun en pueblos rurales.

Si bien la distribución por edades sigue acusando desplazamiento todavía se observan las tasas más altas en el segundo año de la vida

el 80 % de los casos en el primer trienio y el 90 % en el primer quinquenio. Entre los niños mayores y los adultos la enfermedad continua siendo manifiestamente excepcional. Se nota predilección por el sexo masculino. La denuncia es siempre defectuosa y el 70 % de los pacientes corresponde a niños hospitalizados frente a un 2 % de notificaciones provenientes de médicos particulares. Se advierte mejor conocimiento de las formas no paráliticas y abortadas.

El Instituto Bacteriológico dispone de laboratorio bien equipado que está en manos de técnicos competentes y experimentados. Han logrado ellos el aislamiento y el cultivo de unas 218 cepas de virus de polio. Su mayoría corresponde a los tres tipos antigenicos mejor conocidos y se distribuyen a su vez en un 80 % para el Brunhilde, 15 % para Lansing y 5 % para Leon. Uno que otro presenta variaciones. Las epidemias han sido causadas en su gran mayoría por el tipo 1. Se ha encontrado frecuencia apreciable de virus Cocksackie y menor de ECHO que representa aproximadamente un 15 % de los hallazgos. Los estudios serológicos indican que a los cinco años de edad más del 50 % de los individuos tienen anticuerpos para uno o más viruspolio. En frecuencia decreciente corresponde a los tipos 2 y 3.

Diversas partidas de vacuna Salk han sido importadas por empresas y personas particulares y su empleo no ha dado lugar a ningún contra tiempo. Ahora el Servicio Nacional de Salud se ha encargado de su importación en escala mayor y la está ofreciendo al público en sus Centros de Salud y a precio de costo. La entregará gratuitamente a los indigentes. Además el Servicio

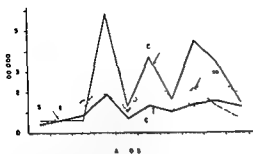


FIG 13 Mortalidad por poliomielitis en Valparaíso Santiago Concepción y Chile 1947-1956

practicará vacunaciones sistemáticas en una ciudad (Concepción) con po o mas de 100 000 habitantes con el objeto de estudiar las diferencias entre grupos vacunados y no vacunados. En atención a la distribución por edades observada está recomendando inmunizar a los niños menores de cinco años. La existencia de problemas sanitarios de mayor envergadura no le permite todavía realizar esta empresa en gran escala. Se está considerando seriamente la posibilidad de prepararla en Chile y tanto los estudios como las gestiones necesarios para el propósito están avanzados.

La feliz circunstancia de que el Servicio Nacional de Salud dispone de la gran mayoría de las camas hospitalarias del país ha permitido disponer del número requerido para los casos de esta enfermedad y dar facilidades especiales para su traslado. Se cuenta con número suficiente de aparatos de respiración artificial. En cambio las dotaciones para rehabilitación y el personal respectivo son todavía insuficientes.

Una ley está próxima a conceder fondos especiales para desarrollar la rehabilitación. Esta actividad quedará naturalmente incorporada en el Servicio Nacional de Salud, pero los reglamentos respectivos son suficientemente elásticos como para permitir su administración expedita. Habrá departamentos de medicina física y rehabilitación en todos los hospitales de 200 o más camas y uno o más institutos con dotación mayor y fabricación de aparatos ortopédicos. La necesidad de desarrollar esta especialidad se deja sentir agudamente y constituye preocupación preferente de algunas autoridades.

Las características epidemiológicas de la poliomielitis en Chile nos hacen suponer que la vacu-

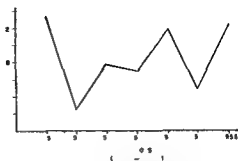


FIG 14 Morbilidad por poliomielitis en Chile 1950-1956

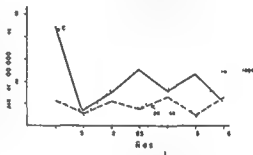


FIG 15 Morbilidad por poliomielitis en Santiago Valparaíso y Concepción 1950-1956

nación pueda ser particularmente eficaz. Nuestras colectividades están perdiendo su inmunidad ante nuestros ojos y se podría suponer que estamos en el momento preciso en que se la puede estimular considerablemente con el empleo de un antígeno de eficiencia aun relativa. Nuestra máquina administrativa permite llevar a la práctica una medida de esta especie y las enseñanzas que pueda aportar habrán de ser útiles para otros países de condiciones similares.

## CZECHOSLOVAKIA

DR. VILÉM ŠKOVRAŇEK

### EPIDEMIOLOGISCHE SITUATION

Polioepidemien treten in der CSR seit dem Jahr 1939 auf. Seit dieser Zeit wiederholen sich Polioepidemien in Intervallen von 4 bis 5 Jahren. Die durchschnittliche ganzstaatliche Morbidität erreichte in epidemischen Perioden ca. 20 Kranke auf 100 000 Einwohner in aussergewöhnlichen Perioden 4 bis 8 Kranke auf 100 000 Einwohner (falls die ausserordentlich niedrige Morbidität

des Jahres 1955 nicht in Betracht gezogen wird) Die Entwicklung der Morbidität vom Jahr 1939 bis zum Jahr 1956 demonstriert am besten die graphische Veranschaulichung auf der sehr gut die zwei letzten Epidemien (1948 und 1953) ersichtlich sind Die Morbidität ist aber beim Auftreten von Epidemien nicht gleich massig auf das ganze Staatsgebiet verlagert sie unterscheidet sich grundlegend in verschiedenen Staatsgebieten während die Morbidität an manchen Orten die Ziffer von 50 bis 80 Kranken auf 100 000 Einwohner überschreitet ist sie an

anderen Orten nur gering Dies demonstriert sehr gut wenigstens ein Kartogramm aus der Epidemie 1953 Was die Altersinzidenz anbelangt ist das Maximum der Krankenzahl bisher bei Kindern im vorschulpflichtigen Alter wenn auch fortlaufend eine Verschiebung der Morbidität auch in einige höhere Jahrgänge zu beobachten ist Auf dem Graph der Altersinzidenz ist dies gut ersichtlich Dabei besteht ein gewisser Unterschied zwischen den westlichen (Böhmen und Mähren) und östlichen (Slowakei) Staatsgebieten was durch die abweichende historische und gesellschaftliche Entwicklung dieser Staatsgebiete erklärt werden kann Die fortlaufende Verschiebung der Morbidität in

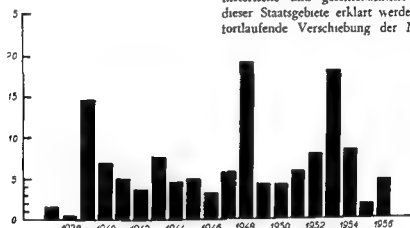


FIG 16 Polomyelitis in Czechoslovakia 1937-1956 (notified cases per 100 000 inhabitants)

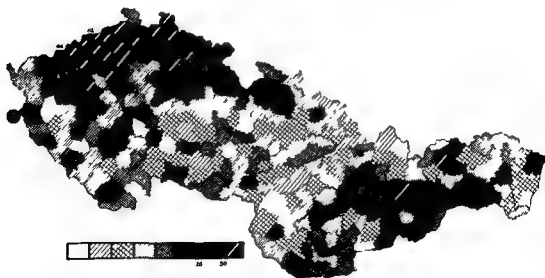


FIG 17 Polomyelitis in Czechoslovakia in 1953 (per 100 000 inhabitants in different districts)

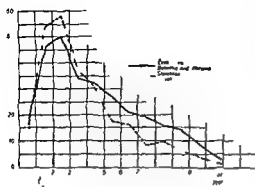


FIG. 18 Age group morbidity (average of the years 1951-1955 per 100 000 inhabitants)

einige höheren Altersgruppen demonstriert ein weiterer Graph auf dem zum Vergleich die Änderungen in der Morbidität vom Jahr 1937 bis zum Jahr 1952 angeführt sind. Die epidemiologischen Analysen die in der CSR dokumentiert schon eine Reihe von Jahren durchge-

führt werden vervollständigen nunmehr auch ausführliche virologische und immunologische Analysen. Die Altersinzidenz entspricht zu einem gewissen Masse auch den serologischen Befunden die vom Immunologischen Forschungsinstitut in Prag (Dr. Zacek) durchgeführt wurden.

### KURZ ÜBER DIE HEILBETREUUNG VON ERKRANKTEN

In der CSR ist für jeden Poliokranken eine unentgeltliche Heilbetreuung und Nachbehandlung seitens des Staates sichergestellt. In jedem Kreis unseres Staates sind spezielle Heilzentren für Polio errichtet. Ausserdem bestehen zwei gut eingerichtete Institute für Nachbehandlung in Kurbadern (Johannsbad und Gross-Lossin).

### ZUR VAKZINATION GEGEN POLIO

Die epidemiologische Situation in der CSR hat uns klar vor die Notwendigkeit gestellt die Erfahrungen amerikanischer wissenschaftlicher Fachleute für eine Polioprävention auch in der

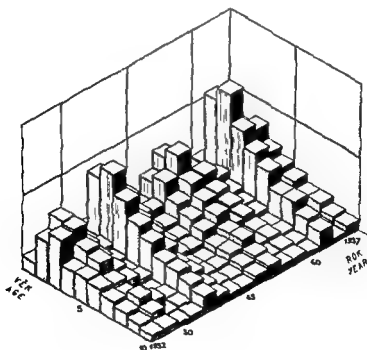
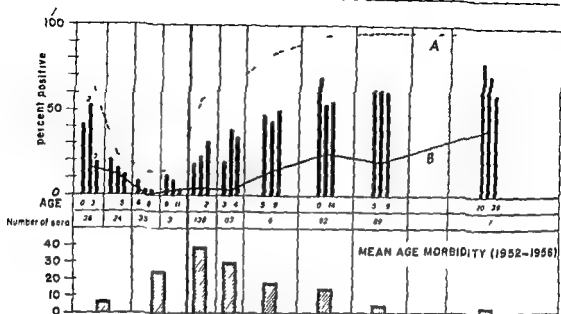


FIG. 19 Age group morbidity in Bohemia and Moravia (per 100 000 inhabitants)

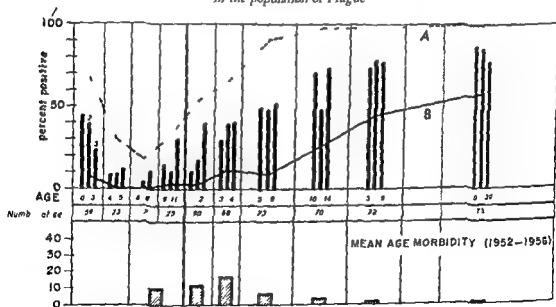


(778 examined sera were collected during first 3 months of 1957)

A Antibodies to any 1 type

B Antibodies to all 3 types

FIG 20 Distribution of neutralizing antibodies against all 3 types of poliomyelitis viruses in the population of Prague



(602 examined sera were collected during first 4 months of 1957)

A Antibodies to any 1 type

B Antibodies to all 3 types

FIG 21 Distribution of neutralizing antibodies against all 3 types of poliomyelitis viruses in the population of Jihlava (a rural district)

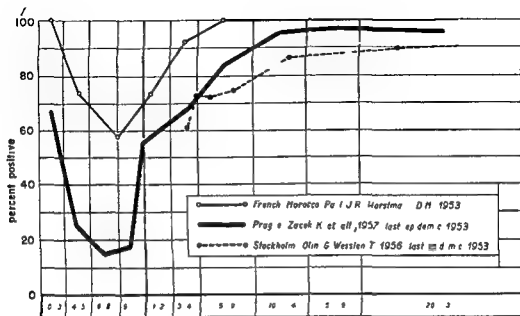


FIG 27 Neutralizing antibodies to any one type of poliomyelitis virus

AGE

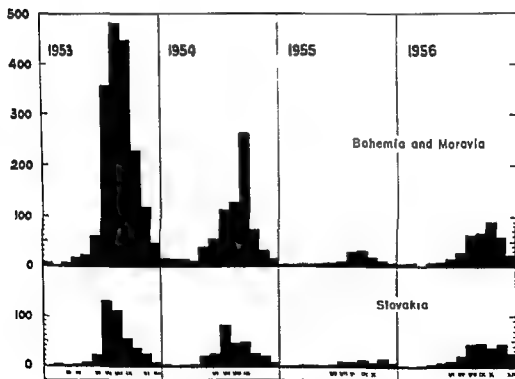


FIG 23 Poliomyelitis in Czechoslovakia 1953-1956

CSR auszunutzen. Es wurden deshalb alle Vorbe-  
reitungen für die Herstellung eines Polioimpf-  
stoffes noch in diesem Jahr getroffen. Unsere  
ursprüngliche Voraussetzung—Ende 1957 und  
zu Beginn 1958 zu impfen—wurde durch die  
atypische Poliomorbidität im Jahr 1956 abge-  
ändert. Obwohl es zu keinem besonders hohen  
Auftreten kam, war die Morbiditätsverschiebung  
bis in die späten Jahresmonate verbluffend. Dies  
demonstriert gut ein Graph, auf dem die Mor-  
bidität vom Jahr 1953 bis zum Jahr 1956 ver-  
anschaulicht ist. Ende des Jahres 1956 wurde  
deshalb im Ausland der Impfstoff bestellt und  
alle organisatorischen Massnahmen zur Einlei-  
tung der Impfkation getroffen. Infolge etwas  
verspäteter Lieferung des Impfstoffes konnte  
mit der Aktion erst am 2. Mai 1957 begonnen  
werden, wo sich die epidemiologische Situation  
im Vergleich mit dem Jahr 1956 schon sehr  
wesentlich verändert hatte. Wir standen klar  
im Entfaltungstadium einer Epidemie, was  
gut aus einem Graph ersichtlich ist, wo die Mor-  
bidität nach Wochen angeführt ist (oben das  
Jahr 1956, unten das Jahr 1957). Trotzdem  
entschieden wir uns zu impfen. Wir erläuterten  
der Bevölkerung die Möglichkeit zeitlicher  
Koinzidenz zwischen Impfung und eventueller  
Erkrankung. Ich kann hier erklären, dass  
unsere Bevölkerung die Impfkation mit grossem  
Verständnis aufgenommen hat. Ein Beleg dafür  
ist auch die hohe Impfbeteiligung. Im Laufe

der Monate Mai und Juni wurden [mit zwei  
Injektionen] insgesamt 2,318 074 Kinder ge-  
impft, davon mit zwei Injektionen 2,235 000,  
welche Zahl ungefähr 18 % der Gesamtbe-  
völkerung der CSR darstellt und Kinder im  
Alter von 6 Monaten bis zu 7 Jahren in der  
Mehrzahl der Städte noch Kinder bis zu 11  
bzw. bis zu 13 Jahren einschliesst.

Zur Organisation der Impfkation möchte ich  
anführen, dass sie zentral vom Ministerium für  
Gesundheitswesen in den Kreisen und Bezirken  
von den Kreis- und Bezirkshygienikern geleitet  
wurde. Mit der Durchführung der Impfung  
waren Aerzte des normalen Netzes des Gesun-  
heitsschutzes vor allem Kinderärzte betraut,  
wobei bei der Impfung freiwillige Arbeiter des  
Tschechoslowakischen Roten Kreuzes behilflich  
waren. Eine ausführliche klinische und viro-  
logische Untersuchung jedes Falles von Er-  
krankungsverdacht ist sichergestellt. Bei der  
Impfkation kam es zu keinen ernstesten Kom-  
plikationen. Auf Grund dänischer Erfahrungen  
wurde die Impfung mittels intrakutaner Tech-  
nik durchgeführt. Verabfolgt wurden immer  
zwei Injektionen zu je 0.1 bis 0.15 ml, also in  
jedem Fall durchschnittlich 0.25 ml Impf-  
stoffes. Dies war einerseits durch einen gewissen  
Mangel an Impfstoff bedingt, andererseits  
durch den Umstand, dass bei der hohen gesund-  
heitlichen Reife unserer Bevölkerung nur  
schwerlich eine bloss experimentelle Impfung

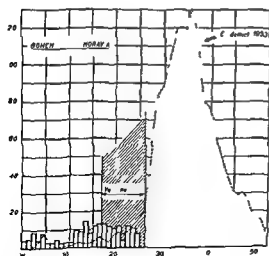


FIG 24 Cases of paralytic poliomyelitis in Bohemia and Moravia in 1957

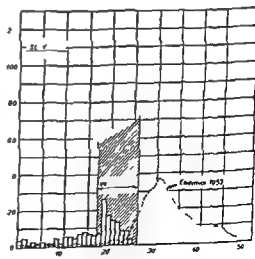


FIG 25 Cases of paralytic poliomyelitis in Slovakia in 1957

von beschränkten Kindergruppen hatte erklärt werden können. Ausserdem haben wir vorausgesetzt, dass durch den positiven Einfluss einer Massensimpfung die durch kollektive Immunität gewisse Mängel in der individuellen Immunität überbrückt werden können.

Die Ergebnisse der Aktion, die erst am 1 Juli abgeschlossen wurde, können begreiflicherweise noch nicht bewertet werden. Ich kann nur die Schnelligkeit der Aktion bewerten, die von unseren Ärzten wirklich vorbildlich durchgeführt wurde.

Die Entwicklung der Morbidität während der Aktion ist aber trotzdem interessant, sodass ich mir schliesslich doch gestatte sie zu demonstrieren.

Sollten sich unsere Voraussetzungen über den Einfluss der kollektiven Immunität bewähren, könnten unsere umfangreiche und zeitlich rasch durchgeführte Aktion in den nächsten Monaten und Jahren die epidemiologische Polio-situation der CSR beeinflussen.

## DENMARK

### DR E. JUEL HENNINGSEN

Since the 2 severe outbreaks of polio in 1952 and 1953 comprising more than 3 000 paralytic cases which I described at the Third International Poliomyelitis Conference in 1954, Denmark has been spared epidemic polio. Only sporadic cases have occurred: there were 72 cases in 1954, 24 in 1955 and 37 in 1956.

The after-treatment and the rehabilitation of the many patients with paralysis have placed a great responsibility on the public hospital system, the Danish National Association for Infantile Paralysis and the Society and Home for Cripples.

In Blegdamshospital, Copenhagen, 70 patients with respiratory piroses are still hospitalized. A special institution for these chronic patients is being built. A few patients have been moved to their own homes and given complete practical and financial support from private and public authorities.

The great event in the fight against poliomyelitis in Denmark has been the introduction of polio vaccination. When Salk published his first studies on polio vaccine in 1952, the Danish State Serum Institute (Statens Seruminstitut) was prepared to take up experimental work on

this line. The scientists in the Institute had followed closely the work in Salk's laboratory. After the Third International Polio Conference in Rome in September 1954, it was decided to start a Danish polio vaccine production. The polio vaccine was manufactured at the Danish State Serum Institute and was prepared according to Salk's method. No other vaccine has been used in Denmark.

On April 17, 1955, when Dr. Thomas Francis, Jr. published the report "Evaluation of 1954 Field Trial of Poliomyelitis Vaccine," the Danish State Serum Institute had vaccine in stock for about 400 000 children. The National Health Service decided that 400 000 school children from the first 5 grades (7 to 12 years of age) should be the first to be vaccinated.

After careful consideration, the following principles for vaccination were established:

1. The vaccination should be entirely voluntary and permission obtained from the parents before vaccination.

2. The children were to be given 3 injections—the first and the second with about a 4 week interval, the third about 12 months later.

3. At each injection the child should be given 2 intradermal injections in the forearm, the total amount of vaccine employed being about 0.3 ml.

The vaccination of these 400 000 school children was started on April 20, 1955, not more than a week after the publication of the American report.

The program was organized by the country's 66 medical officers of health in collaboration with the local branches of the Danish Medical Association. About 600 school physicians and specially appointed general practitioners carried through the campaign, which included more than 98 per cent of the school children in the first 5 classes in 4 000 schools in Denmark. The campaign was completed without any accidents.

A special antipoliomyelitis vaccination act was passed by Parliament on May 24, 1955. In consequence of this act, vaccination is voluntary and free of charge for everybody under 40 years. Vaccination is performed by all general practitioners who receive their fees from the State. This is in accordance with the general principles followed in Denmark for prophylactic health measures.

The amount of vaccine ready for use in April



1955 was not sufficient for immediate vaccination of the 2 700 000 inhabitants under 40 years of age. The Minister of the Interior in accordance with the amount of vaccine available has decided when the different age groups should be allowed to be vaccinated. Children under school age were vaccinated in the fall of 1955 in the spring of 1956 all persons up to 25 years of age were permitted vaccination. In June 1956 all persons under 40 years of age were allowed vaccination.

To what extent has the population made use of this service of the Government of Denmark?

Ninety nine per cent of children between 9 months and 14 years of age have been vaccinated against polio and no difference between numbers of children vaccinated in the capital the provincial towns and the rural areas has been observed.

Ninety three per cent in the age group 15 to 19 and 85 per cent between 20 to 39 years have been vaccinated and again there is no difference between the capital and the provincial towns though in the rural areas the figures are slightly smaller. It can be stated moreover that no difference between rich and poor was found.

Since September 1956 anyone over 40 years of age may be vaccinated at his own request as expected only relatively few have asked for vaccination.

The figures which are increasing daily show that nearly 100 per cent of the Danish population in the age groups especially exposed to polio are vaccinated. More than 2,500 000 out of a population of 4 500 000 have already benefited from the polio vaccination.

In order to follow the antibody response blood specimens from about 1500 school children were taken immediately before the first injection and about 1 month after. The serologic results have shown that the vaccination has had the anticipated effect.

As I mentioned earlier Denmark has had only few sporadic cases of poliomyelitis during the last 3 years. I want to stress that we have not drawn any conclusions from this as to the effect of the vaccine. In this connection it must be remembered that the high percentage of vaccinations in the population means that there is no control group in the scientific sense of the word.

Denmark has had no real vaccine surplus

during these years. However it has been possible to make vaccine available to Iceland as well as to Norway. In these two countries 400 000 persons have been vaccinated with Danish vaccine.

You may recall that with the background of the big poliomyelitis epidemics in Norway and Denmark a Scandinavian Poliomyelitis Board was appointed in 1953. The collaboration among the Scandinavian countries has resulted in many valuable negotiations through these years and practical assistance was given to Iceland during a severe outbreak of poliomyelitis in 1955.

On behalf of the Scandinavian Poliomyelitis Board and on behalf of my own country I have the honor to greet the Fourth International Poliomyelitis Conference and to express our gratitude to our Swiss hosts and to the National Foundation for Infantile Paralysis.

## EGYPT

PROF M DIWANY

In Egypt poliomyelitis is not a new disease. Ancient Egyptian sculptors have left us a relief dating from the 18th Dynasty (about 1500 B.C.) in which the priest Ruma is shown with an atrophic shortened leg and a paralytic pointed foot suggesting that the man obviously had suffered from spinal poliomyelitis.

In spite of this historical evidence poliomyelitis was considered to be a rare and interesting disease at the time when I first joined the University Children's Hospital at Cairo in 1931. Since then poliomyelitis has shown a progressive increase in incidence and a change from a sporadic occurrence to an endemic type spread. In the year 1939 for example the number of cases referred to the Electrotherapy Department of our Hospital was only 38 whereas in 1953 there were 889 cases i.e. more than a twenty threefold increase in a period of 15 years. In 1955 there were 1130 cases and in 1956 757 cases. If it is considered on a very modest scale that an equal number is also treated in other hospitals and private clinics the total incidence of polio in 1953 can be assumed as exceeding 1800 cases making a case rate of 9 per 100 000. The average case rate in the U.S.A. for 1932 to 1946 is 7.26 per 100 000.

TABLE 5 SEASONAL INCIDENCE OF DIFFERENT TYPES OF POLIO

TOTAL No	1955												1956	
	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC	JAN	FEB
	63	67	48	75	106	133	86	112	123	126	126	94	115	59
Type 1	NST	1	0	0	2	7	10	5	NST	0	3	11	4	1
Type 2	NST	2	1	2	3	13	16	1	NST	4	2	3	2	0
Type 3	NST	2	1	0	0	4	4	3	NST	1	1	2	0	1

NST = Not possible to type

In Egypt poliomyelitis has not broken loose from its age limitation to early childhood. Most of the cases are under 2 years of age, the highest peak being between 10 and 16 months. Males are affected more than females in the proportion of 3 to 2. A seasonal variation in incidence is not striking—cases are encountered throughout the year with some monthly fluctuation. The spinal form of the disease is the commonest form met. Lower limbs are affected more frequently than upper limbs and both upper and lower extremities more than the trunk. The incidence of bulbar polio is less than 1 per cent in contrast to more than 6 per cent abroad. This may be explained by the earlier age incidence in our country and the fact that tonsillectomy usually is performed after the third year of life. There has been observed among our infants and children a high incidence of isolated 7th nerve paralysis which is probably of polio virus etiology.

The work of Paul *et al.* carried out in Egypt in 1950, 1951 and 1952 throws much light on the question of age incidence. Neutralizing antibodies to the Lansing, the Leon and the Brunhilde types were found to be acquired at an early age among Egyptians. With improvement of hygienic and social standards we expect the disease to affect older age groups and to approach the pattern observed today in the U.S.A. and in Europe.

Polio virus studies were made in our department in collaboration with the United States Naval Medical Research Unit No. 3 under the supervision of Dr I. Taylor, now Professor of Virology at Yale University and with the help of Dr D. Horstmann, Associate Professor of Virology at Yale University.

In the series studied specimens for isolation of the virus were taken only from patients in the first 14 days of the illness. Monkey kidney or HeLa cells were used for isolation but for typing only monkey kidney cells were used. It was found that Type 2 exceeded both Types 1 and 3 in incidence giving a total incidence of 44 per cent. Types 1 and 3 gave an incidence of 39 per cent and 17 per cent respectively. It was also observed that the domination of Type 2 (Type 2 48.6% of cases Type 1 33.4% Type 3 16.6%) was more marked during the hot months while Type 1 (Type 1 63.3% of cases Type 2 23.3% Type 3 13.3%) was more frequent during the cooler months (Table 5). As regards the age incidence the youngest child from whom the polio virus was isolated was 3½ months old and the oldest was 5 years old. Type 1 was predominant below 2½ years after which the other types prevailed.

The 100 per cent isolations in this series were almost reached at 2½ years for Type 1 at 4 years for Type 2 and at 5 years for Type 3. The most extensive paralysis was found in patients infected with Type 2 virus, the extent of paralysis with the other 2 types being about equal (Table 6).

Compared with the U.S.A., Holland and South Africa where Type 1 is strictly predominant in Egypt Type 2 exceeds both Type 1 and Type 3 (Table 7). According to Paul *et al.* in the isolations that were made in Egypt in 1943, 3 out of 7 strains were Type 2 polio virus, i.e., 40 per cent. Also serologic studies done by Paul in Egypt in 1950 among normal infants and children revealed that Type 1 polio virus antibodies lag behind Type 2 antibodies.

TABLE 6 RELATION BETWEEN TYPE OF VIRUS ISOLATED AND EXTENT OF PARALYSIS

TYPE	1 EXTREMITY	2 EXTREMITIES	2 EXTREMITIES AND TRUNK OR 3 EXTREMITIES	TRUNK AND 4 EXTREMITIES (AND DIAPHRAGM)
1 (47 cases)	37 (79%)	8 (15%)	1	1
2 (53 cases)	31 (59%)	15 (30%)	4	3
3 (22 cases)	17 (77%)	4 (19%)	1	1

TABLE 7 PREDOMINANCE OF TYPE 2 VIRUS IN EGYPT AS COMPARED WITH OTHER COUNTRIES

TYPE	EGYPT (1955)	U S A (1953)	HOLLAND (1952)	SOUTH AFRICA (1954)
1	39%	80%	68%	42%
2	44%	6%	28%	33%
3	17%	14%	4%	23%

In Egypt the Coxsackie virus is quite common and in some instances may be associated with viremia. It has been accidentally isolated from blood at the United States Naval Medical Research Unit No. 3 on 19 occasions and frequently was isolated from stools, oropharyngeal secretions and flies. Types B-2, B-4, A-2, A-3, A-4, A-6 and A-8 were isolated from Egyptian children.

It is important to mention that ECHO virus Prototype 1 was isolated in 1954 from an Egyptian child in Cairo by Dr. J. Melnick. The child was apparently normal and asymptomatic. Dr. Horstmann examined rectal swabs from 200 asymptomatic Egyptian children and out of these 96, i.e. 48 per cent, yielded orphan viruses. In a previous work done in 1952, Dr. Horstmann found that out of 300 children examined, 126 isolations were orphans, i.e. 42 per cent.

Could the orphan viruses, by being spread so widely in our community among our children in an asymptomatic fashion or with only minor symptoms, be cross-immunizing us against some other more vicious viruses in the same way that the West Nile virus is rendering us cross-immunized to some of the other encephalitis viruses?

Should we ignore a virus simply because it is associated with a mild ailment or even none? Mutation may convert a relatively harmless or

ganism into something capable of causing a lethal infection.

Probably there is a relation between viruses of different types and the appearance of paralysis in some cases when no neurotropic virus was isolated, indicates that under certain circumstances a comparatively benign organism may somehow acquire unseen destructiveness. It is possible that a person with either passive or active immunity to the 3 conventional types of polio may still be susceptible to a polioli-like illness, paralytic or nonparalytic. And conversely, a person infected with any of these viruses may be mistakenly believed to be immune to polio.

## FINLAND

### DR. M. E. PARVIALA

In Finland so far poliomyelitis has not taken the form of an alarming epidemic. In 1945, 1954 and 1956 the worst years in this respect, the number of reported clinical cases amounted to about 18 to 20 per 100,000 inhabitants. However, it has been discovered in laboratory tests for poliomyelitis neutralizing antibodies that the mean immunity is 50, 70 and 90 per cent at the ages of 5, 20 and 40 respectively. Subclinical infection thus is fairly common.

About 75 to 80 per cent of the reported clinical cases involve paralysis and about 12 to

15 per cent of these patients suffer from respiratory paralysis.

Paralytic cases are attended to at the epidemic wards of rural communal hospitals or at home or in the bigger towns at special hospitals for communicable diseases. Only the latter have the necessary facilities for giving rehabilitation treatment at an early stage. If it proves impossible to arrange such treatment and if orthopedic surgery is required the patient is transferred to the State Invalid Hospital or to the Orthopedic Hospitals or the Rehabilitation Institutes run by private foundations and organizations. As these hospitals also treat other patients who are in need of orthopedic treatment or rehabilitation the number of places available for polio cases at present is limited. To meet the needs of northern Finland the building of a new State-owned invalid hospital is under consideration.

The treatment of respiratory paralysis to the largest possible extent has been concentrated in the 3 largest hospitals for communicable diseases in the cities of Helsinki, Turku and Tampere. These hospitals have trained staffs and the necessary respirators at their disposal. In addition a center containing 6 to 7 beds is now being equipped at the Provincial Hospital of the town of Oulu to cater to acute cases in the north of Finland. For patients suffering from chronic respiratory paralysis (the present number in Finland is about 20) the State center with 12 to 15 beds will be ready in the near future. Since the treatment of respiratory paralysis incurs great expenses—annually some 3 to 5 million marks per patient—individual communities have been unable to cover hospital expenses from their funds. Such costs also being beyond private means the necessary financial assistance has been applied for from the State funds. In the beginning of 1957 a new law went into effect according to which the State pays all acceptable expenses incurred from the treatment of respiratory paralysis.

The transportation of the above mentioned polio patients is a difficult problem in Finland. The majority of the population lives in the southern and the southwestern parts of the country whereas the eastern and the northern parts are populated sparsely and the network of roads is insufficient. Since cases of respiratory paralysis call for urgent transportation it is as

a rule necessary to use air transport. Our large waterways offer suitable landing places for sea planes and in the winter when the lakes are icebound planes provided with skis can land easily. But during the spring thaw as well as in the autumn before freezing air transport cannot be used. Therefore the purchasing of a sufficient number of helicopters appropriate for such transport is under consideration.

Another difficulty arises from the shortage of fully trained nursing staffs. As the number of hospital nurses is inadequate in general and as the nursing of patients suffering from respiratory paralysis is especially strenuous both physically and mentally the fully trained personnel prefers easier employment. The situation may become easier in this respect as there are good hopes that the gap in the nursing profession will be gradually filled in during the course of the next few years.

Vaccination against polio has been going on since 1954. The first vaccinations were of an experimental character and they were performed on a small scale in certain districts earmarked for this purpose. As of spring 1957 mass vaccination involving about 150 000 children was started throughout the country. The object is to carry on vaccination and to increase the number of children eligible for it so that finally all age groups which are susceptible to polio will be included in this scheme.

## FRANCE

### DR. LACOMBE

Au cours des 3 années qui ont suivi le précédent Congrès la fréquence de la poliomyélite n'a pas marqué de variations notables en France.

Si la maladie y marque depuis 1917 date à laquelle fut instituée l'obligation de sa déclaration, une progression continue celle-ci reste relativement lente. Rappelons que la moyenne annuelle des cas déclarés de 700 pour la période 1917-1929 est passée à 500 pour la période 1930-1947 et à 1 400 pour la période 1943 à 1953. L'indice moyen de morbidité pour cette dernière période étant de 34.

1950 reste l'année où fut enregistrée la plus forte poussée de poliomyélite en France avec 1 979 cas déclarés et un indice de morbidité relevant à 47.

La France garde en ce qui concerne la polio-

myélite une situation assez privilégiée et cela même par rapport à la plupart des pays limitrophes de son territoire

Le tableau 8 comporte l'indication des totaux annuels de cas déclarés et des indices annuels de morbidité enregistrés en France depuis 1954

**TABEAU 8 LES TOTAUX ANNUELS DE CAS DÉCLARÉS ET LES INDICES ANNUELS DE MORBIDITÉ ENREGISTRÉS DEPUIS 1954**

ANNÉES	NOMBRE DE CAS	INDICES DE MORBIDITÉ RAPPORTÉS 100 000 HABITANTS
1954	1 534	3 5
1955	1 834	4 2
1956	1 150	2 6

A noter que les déclarations concernent essentiellement des formes paralytiques

Calculés à partir de ces chiffres le nombre moyen de cas déclarés au cours de ces 3 années qui s'élève à 1 500 et l'indice moyen de morbidité qui est de 3,4 apparaissent très voisins de ceux enregistrés pour la période antérieure

Il convient toutefois de signaler une certaine recrudescence de la poliomyélite au cours des premiers mois de 1957 (651 cas déclarés pour les 25 premières semaines contre 259 pour la période correspondante de 1956 mais le total de 1956 était le plus bas enregistré depuis 1948)

L'étude de la répartition des cas par âge montre toujours une prédominance très marquée de la maladie dans les tranches d'âge inférieures au cours des 3 années en cause 36 % des cas sont survenus de 0 à 4 ans 31 % de 5 à 14 ans et 33 % au dessus de 15 ans taux sensiblement comparables à ceux observés pour la période précédente (35 % / 28 % et 37 %)

En ce qui concerne la répartition des cas par sexe l'androtropisme de la maladie reste net. Pour les 3 années en cause 57 % des cas ont été masculins contre 43 % de cas féminins taux identiques à ceux observés pendant la période précédente

Si la morbidité poliomyélitique n'a pas marqué dans ces dernières années de variations notables on note par contre une diminution de la mortalité

En effet alors que pour la période 1943-1953 le nombre moyen annuel des décès était de 236

l'indice moyen de mortalité (nombre de décès par poliomyélite pour 100 000 habitants) de 0,6 et le taux de létalité (nombre de décès pour 100 cas de la maladie) de 16 pour la période 1954-1956 le nombre moyen annuel de décès n'est que de 146 l'indice moyen de mortalité de 0,3 et le taux de létalité de 9,6

**TABEAU 9 L'INDICE DE MORTALITÉ 1954-1956**

ANNÉES	NOMBRE DE DÉCÈS DÉCLARÉS	INDICE DE MORTALITÉ	TAUX DE LÉTALITÉ
1954	141	0 3	9 1
1955	184	0 4	10 0
1956	114	0 27	9 9

Il semble qu'on soit en droit d'établir un rapport entre cette régression de la mortalité et les efforts faits pour améliorer les conditions de traitement des formes aiguës de la maladie — notamment des formes respiratoires

Le nombre des décès reste plus élevé dans le sexe masculin que dans le sexe féminin et les chances de survie sont plus limitées à l'âge adulte que dans l'enfance

La maladie garde en France une allure endémique sporadique avec une recrudescence saisonnière

L'augmentation de la durée de la poussée saisonnière annuelle déjà signalée lors du précédent Congrès s'est précisée encore dans les 3 dernières années la recrudescence annuelle se prolongeant maintenant pendant 7 mois. Cette poussée saisonnière se manifeste de façon plus intense dans certaines zones de localisation géographique très variable d'une année à l'autre

Ainsi en 1954 c'est le département du Morbihan en Bretagne qui fut le plus atteint en 1955 un département du Sud-Ouest la Corrèze et en 1956 trois départements méridionaux l'Ariège l'Hérault et la Haute-Garonne. L'extension de la maladie dans la moitié sud du territoire d'aujourd'hui indiquée dans le rapport précédent s'est donc confirmée au cours des 3 années écoulées

En vue de tenter d'approfondir les connaissances sur l'épidémiologie de la poliomyélite des recherches immunologiques sont poursuivies en France depuis plusieurs années. Elles ont été entreprises en premier lieu par le Laboratoire des Virus de l'Institut Pasteur de Paris

Depuis 1954 en outre le Laboratoire de Virologie de la Société d'Etudes et de Soins pour les enfants poliomyélitiques (SESEP) poursuit également des recherches de cet ordre avec l'aide de l'Institut National d'Hygiène.

A signaler également les examens pratiques par le Laboratoire d'Hygiène de la Faculté de Médecine de Lyon avec l'aide de l'Institut National d'Hygiène et de la Caisse Nationale de Sécurité Sociale.

Ces travaux ont pour objectifs essentiels

1° De chercher à déterminer la courbe d'acquisition des anticorps en fonction de l'âge dans certains échantillons de la population française.

Une enquête a été menée à cet égard par l'Institut Pasteur de Paris à partir des sérums prélevés chez 357 enfants de 20 mois à 21 ans confiés au Service de l'Assistance Publique de Paris (enfants d'un milieu social modeste).

L'autre est effectuée par le Laboratoire de la Société d'Etudes et de Soins pour les enfants poliomyélitiques à partir des sérums prélevés chez 546 enfants hospitalisés dans les villes de Tours, Le Havre, Rouen et Nantes.

Une troisième enquête qui a déjà porté sur plus de 700 sérums est poursuivie par le Laboratoire d'Hygiène de la Faculté de Médecine de Lyon. Les sérums examinés proviennent d'une part d'enfants originaires de toutes les régions de la France en groupes dans une même collectivité et d'autre part d'enfants vivant en milieu rural.

Les résultats obtenus à la suite des 2 premières enquêtes sont sensiblement comparables.

(A) Entre 2 ans et 2½ ans dans les 2 groupes examinés 50 % des enfants possèdent au moins 1 des 3 types d'anticorps.

(B) Dans le groupe d'âge 7—9 ans l'Institut Pasteur trouve 40 % des sujets possédant les 3 types d'anticorps. Dans le groupe d'âge de 7 ans 50 % des enfants sont trouvés porteurs de 3 types d'anticorps par le Laboratoire de la Société d'Etudes et de Soins pour les enfants poliomyélitiques.

(C) Entre 19 et 21 ans 77 % des sujets examinés par le Laboratoire de l'Institut Pasteur possèdent les 3 types d'anticorps.

Les enfants de 15 ans examinés par le Laboratoire de la Société d'Etudes et de Soins pour les enfants poliomyélitiques présentent dans la proportion de 70 % des anticorps aux 3 types de virus.

Les résultats obtenus par le Laboratoire d'Hygiène de la Faculté de Médecine de Lyon s'avèrent un peu différents des précédents. Pour les mêmes catégories d'âge la proportion des sujets porteurs des 3 types d'anticorps est inférieure à celles observées précédemment (1) dans le groupe d'âge de 5 à 9 ans 14 % seulement des sujets seraient porteurs des 3 types d'anticorps (2) dans le groupe d'âge de 15 à 19 ans si tous les sujets présentent des anticorps 43 % seulement sont porteurs des 3 types d'anticorps.

Mais il apparaît que les sujets examinés appartiennent à des milieux sociaux économiques différents de ceux des enquêtes précédentes.

2° De tenter de préciser les variations de l'acquisition des types d'anticorps en fonction du lieu et du temps. Les recherches effectuées à cet égard par le Laboratoire de la Société d'Etudes et de Soins pour les enfants poliomyélitiques ont amené à constater qu'entre les années 1954 et 1955 se manifestait pour le même groupe d'âge et dans des agglomérations différentes une régression du pourcentage des porteurs d'anticorps du Croupe 2 alors qu'augmentait celui des porteurs du Type 3. Ce résultat a d'ailleurs été confirmé par les isolements de virus effectués tant par l'Institut Pasteur que par le Laboratoire de la SESEP à partir des matières fécales de poliomyélitiques ou de sujets contacts.

Si entre 1954 et 1955 une prédominance constante du Type 1 a continué à être observée en France on a pu constater aussi une pro-

TABLEAU 10. TYPES ANTIGENIQUES DES DIFFÉRENTES SOUCHES DE VIRUS ISOLÉES À PARTIR DES SELLES DE MALADES POLIOMYELITIQUES HOSPITALISÉS

	TYPE 1	TYPE 2	TYPE 3
Année 1954			
Résultats, Institut Pasteur	39%	50%	9%
Résultats SESEP	70%	21%	9%
Année 1955			
Résultats Institut Pasteur	77%	7%	1%
Résultats SESEP	65%	9%	6%

myélite une situation assez privilégiée et cela même par rapport à la plupart des pays limitrophes de son territoire.

Le tableau 8 comporte l'indication des totaux annuels de cas déclarés et des indices annuels de morbidité enregistrés en France depuis 1954

TABLEAU 8 LES TOTAUX ANNUELS DE CAS DÉCLARÉS ET LES INDICES ANNUELS DE MORBIDITÉ ENREGISTRÉS DEPUIS 1954

ANNÉES	NOMBRE DE CAS	INDICES DE MORBIDITÉ RAPPORTÉS 100 000 HABITANTS
1954	1 534	35
1955	1 834	42
1956	1 150	26

A noter que les déclarations concernent essentiellement des formes paralytiques.

Calculés à partir de ces chiffres le nombre moyen de cas déclarés au cours de ces 3 années qui s'élève à 1 500 et l'indice moyen de morbidité qui est de 34 apparaissent très voisins de ceux enregistrés pour la période antérieure.

Il convient toutefois de signaler une certaine recrudescence de la poliomyélite au cours des premiers mois de 1957 (651 cas déclarés pour les 25 premières semaines contre 259 pour la période correspondante de 1956 mais le total de 1956 était le plus bas enregistré depuis 1948).

L'étude de la répartition des cas par âge montre toujours une prédominance très marquée de la maladie dans les tranches d'âge inférieures au cours des 3 années en cause 36 % des cas sont survenus de 0 à 4 ans 31 % de 5 à 14 ans et 33 % au dessus de 15 ans taux sensiblement comparables à ceux observés pour la période précédente (35 % / 78 % et 37 %).

En ce qui concerne la répartition des cas par sexe l'androtropisme de la maladie reste net. Pour les 3 années en cause 57 % des cas ont été masculins contre 43 % de cas féminins taux identiques à ceux observés pendant la période précédente.

Si la morbidité poliomyélique n'a pas marqué dans ces dernières années de variations notables on note par contre une diminution de la mortalité.

En effet alors que pour la période 1943-1953 le nombre moyen annuel des décès était de 236

l'indice moyen de mortalité (nombre de décès par poliomyélite pour 100 000 habitants) de 0,6 et le taux de létalité (nombre de décès pour 100 cas de la maladie) de 16 pour la période 1954-1956 le nombre moyen annuel de décès n'est que de 146 l'indice moyen de mortalité de 0,3 et le taux de létalité de 9,6.

TABLEAU 9 L'INDICE DE MORTALITÉ 1954-1956

ANNÉES	NOMBRE DE DÉCÈS DÉCLARÉS	INDICE DE MORTALITÉ	TAUX DE LÉTALITÉ
1954	141	0,3	9,1
1955	184	0,4	10,0
1956	114	0,27	9,9

Il semble qu'on soit en droit d'établir un rapport entre cette régression de la mortalité et les efforts faits pour améliorer les conditions de traitement des formes aiguës de la maladie et notamment des formes respiratoires.

Le nombre des décès reste plus élevé dans le sexe masculin que dans le sexe féminin et les chances de survie sont plus limitées à l'âge adulte que dans l'enfance.

La maladie garde en France une allure endémique sporadique avec une recrudescence saisonnière.

L'augmentation de la durée de la poussée saisonnière annuelle déjà signalée lors du précédent Congrès s'est précisée encore dans les 3 dernières années la recrudescence annuelle se prolongeant maintenant pendant 7 mois. Cette poussée saisonnière se manifeste de façon plus intense dans certaines zones de localisation géographique très variable d'une année à l'autre.

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En vue de tenter d'approfondir les connaissances sur l'épidémiologie de la poliomyélite des recherches immunologiques sont poursuivies en France depuis plusieurs années. Elles ont été entreprises en premier lieu par le Laboratoire des Virus de l'Institut Pasteur de Paris.

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Une troisième enquête qui a déjà porté sur plus de 200 sérums est poursuivie par le Laboratoire d'Hygiène de la Faculté de Médecine de Lyon. Les sérums examinés proviennent d'une part d'enfants originaires de toutes les régions de la France et groupés dans une même collectivité et d'autre part d'enfants vivant en milieu rural.

Les résultats obtenus à la suite des 2 premières enquêtes sont sensiblement comparables.

(A) Entre 7 ans et 21 ans dans les 2 groupes examinés 50 % des enfants possèdent au moins 1 des 3 types d'anticorps.

(B) Dans le groupe d'âge 7-9 ans l'Institut Pasteur trouve 40 % des sujets possédant les 3 types d'anticorps. Dans le groupe d'âge de 7 ans 50 % des enfants sont trouvés porteurs de 3 types d'anticorps par le Laboratoire de la Société d'Etudes et de Soins pour les enfants poliomyéliquiques.

(C) Entre 19 et 21 ans 77 % des sujets examinés par le Laboratoire de l'Institut Pasteur possèdent les 3 types d'anticorps. Les enfants de 15 ans examinés par le Laboratoire de la Société d'Etudes et de Soins pour les enfants poliomyéliquiques présentent dans la proportion de 70 % des anticorps aux 3 types de virus.

Les résultats obtenus par le Laboratoire d'Hygiène de la Faculté de Médecine de Lyon s'avèrent un peu différents des précédents. Pour les mêmes catégories d'âge la proportion des sujets porteurs des 3 types d'anticorps est inférieure à celles observées précédemment (1) dans le groupe d'âge de 5 à 9 ans 14 % seulement des sujets seraient porteurs des 3 types d'anticorps (2) dans le groupe d'âge de 15 à 19 ans si tous les sujets présentent des anticorps 43 % seulement sont porteurs des 3 types d'anticorps.

Mais il apparaît que les sujets examinés appartiennent à des milieux sociaux économiques différents de ceux des enquêtes précédentes.

7° De tenter de préciser les variations de l'acquisition des types d'anticorps en fonction du lieu et du temps. Les recherches effectuées à cet égard par le Laboratoire de la Société d'Etudes et de Soins pour les enfants poliomyéliquiques ont amené à constater qu'entre les années 1954 et 1955 se manifestait pour le même groupe d'âge et dans des agglomérations différentes une régression du pourcentage des porteurs d'anticorps du Groupe 2 alors qu'augmentait celui des porteurs du Type 3. Ce résultat a d'ailleurs été confirmé par les isolements de virus effectués tant par l'Institut Pasteur que par le Laboratoire de la SESEP à partir des matières fécales de poliomyéliquiques ou de sujets contacts.

Si entre 1954 et 1955 une prédominance constante du Type 1 a continué à être observée en France on a pu constater aussi une pré-

TABLEAU 10 TYPES ANTIGÉNIQUES DES DIFFÉRENTES SOLCHIES DE VIRUS ISOLÉS À PARTIR DES SÈLES DE MALADES POLIOMYÉLIQUES HOSPITALISÉS

	TYPE 1	TYPE 2	TYPE 3
Année 1954			
Résultats Institut Pasteur	39%	50%	9%
Résultats SESEP	70%	21%	9%
Année 1955			
Résultats Institut Pasteur	72%	7%	1%
Résultats SESEP	65%	9%	



gression sensible du Type 3 s'effectuant surtout aux detriments du Type 2

Sur la base de ces resultats obtenus a partir de prelevements provenant de sujets originaires d'agglomerations differentes il est permis d'avancer que l'evolution des 3 types de virus poliomyelitiques se fait vraisemblablement par poussees successives de grande diffusion

Bien que la poliomyelie ne constitue pas pour la France ainsi qu'on la vu un probleme sanitaire de premier plan des efforts importants ont cependant ete faits au cours des dernieres annees afin de mettre a la disposition de la population les methodes therapeutiques les plus actuelles

1 L'equipement du pays en centres de traitement des formes aiguës de la maladie et specialement des formes respiratoires a ete complete

Etant donne d'une part l'incidence relativement faible de la maladie en France et d'autre part les conditions tres particulieres (tant en ce qui concerne le personnel que le materiel) qui s'imposent pour le fonctionnement de centres de traitement des formes respiratoires de la poliomyelie il est apparu necessaire de ne les creer qu'au sein des principaux centres hospitaliers regionaux

Treize centres specialises repartis sur l'ensemble du territoire ont ainsi ete amenes Ils ont ete dotes du personnel et du materiel specialises necessaires

Afin d'assurer la formation du personnel medical et infirmier affecte a ces centres des stages ont ete organises chaque annee dans les 2 centres de Paris les premiers mis en service

Des dispositions ont ete prises en outre pour que chaque centre regional puisse disposer des differentes varietes d'appareils respirateurs connus poumons d'acier et appareils assurant la respiration par voie endotracheale

Chaque centre regional dessert une circonscription geographique determinee et possede une ambulance qui assure le transport des malades de leur domicile au centre un medecin et une infirmiere affectes a celle-ci peuvent s'il y a lieu dispenser les soins de premiere urgence

2 Dans le domaine de la reeducation fonctionnelle des infirmes poliomyelitiques le developpement des possibilites de reeducation notamment dans les hopitaux publics se poursuit

Pendant cette periode enfin en France comme

dans la plupart des autres pays le probleme de la prophylaxie de la maladie par la vaccination a ete l'objet d'une attention tres particuliere

Un vaccin français inactive a ete mis au point par l'Institut Pasteur de Paris Les souches entrant dans sa composition ont ete selectionnees a partir de 270 souches isolees en France Une des caracteristiques essentielles du vaccin français est l'utilisation pour sa preparation de souches apathogenes des 3 types de virus

Ce vaccin doit etre administre par voie sous cutanee la vaccination comportant 3 injections auxquelles il est conseille d'ajouter l'annee suivante une injection de rappel

La vente du vaccin a ete autorisee depuis le 1er Juin 1956 sur presentation d'une ordonnance medicale

La vaccination qui garde un caractere strictement facultatif peut etre pratquee soit au domicile du medecin praticien soit dans des centres regionaux publics repartis dans les principales villes du territoire

Dans ces centres actuellement au nombre de 15 la vaccination est effectuee gratuitement Elle est precedee et suivie de prelevements sanguins pratiques dans le but de determiner la presence la progression et la persistance des anticorps les examens necessaires etant effectues dans le Laboratoire de Virologie auquel chaque centre de vaccination doit etre rattache

Cette methode est apparue comme celle susceptible de fournir en France les meilleures indications sur la valeur de la vaccination L'incidence de la maladie y est en effet trop faible pour qu'on puisse obtenir des resultats statistiquement valables par la comparaison de la morbidite de groupes de sujets vaccines et de groupes non vaccines Plus de 100 000 vaccinations ont ete pratiquees maintenant en France

## GERMANY

BUNDESREPUBLIK  
DEUTSCHLAND (WEST)

DR HABERNAL

### STAND

Für das Gebiet der Bundesrepublik Deutschland gewinnt die Poliomyelitis seit dem I Weltkrieg zunehmende Bedeutung Die wellenförmige Kurve der Erkrankungen an Kinderlähmung mit den typischen Spätsommergipfeln zeigt im Laufe der Jahrzehnte in Abständen

von 45 Jahren höhere Erhebungen. Die grösste Epidemie ereignete sich im Jahre 1952 mit einer Morbidität von 196 je 10 000 Einwohner. 1956 war ein sogenanntes Normaljahr mit einer Morbidität von 0,79 je 10 000 Lebende. Auffallend war dabei eine Verschiebung der höchsten Krankheitsziffern in den Monat Oktober.

### ALTERSVERTEILUNG

Bei einer breiten Streuung der Erkrankungen bis in das Erwachsenenalter liegt der höchste Anteil der Erkrankungen mit Lahmungen im 2. 3. und 4. Lebensjahr. Untersuchungen zur Aufstellung eines Antikörperkatasters ergaben, dass die Kinder im 2. und 3. Lebensjahr gegen alle 3 Typen nur in wenigen Fällen und nur in durchschnittlich 50% gegen einen der Typen Antikörper besitzen. Die Zahl der Kinder mit Antikörpern nimmt aber mit zunehmendem Alter rasch zu. Bei den Untersuchungen der Zehnjährigen wurden in 80-90% Antikörper festgestellt.

### VERHÄLTNISS DER ERKRANKUNGEN MIT UND OHNE LAHMUNGEN

Über das Verhältnis der Erkrankungen mit und ohne Lahmungen seit 1953 gehen die namentlichen Meldungen der Kinderlähmungsfälle an das Bundesgesundheitsamt Auskunft. Auf 100 diagnostisch gesicherte Erkrankungen entfallen 10,75 Erkrankungen mit Lahmungen.

Die Letalität seit 1953 schwankte zwischen 5,1% und 7,8%.

### BEKÄMPFUNG DER POLIOMYELITIS

Zur Vereinheitlichung der Bekämpfungsmassnahmen wurde 1954 die Deutsche Vereinigung zur Bekämpfung der Kinderlähmung gegründet. In 8 Ausschüssen bearbeitet sie die wissenschaftlichen Probleme der Poliomyelitis (Durch ihre Merkblätter und durch eine Wanderausstellung bemüht sie sich um die Aufklärung der Bevölkerung. In ärztlichen Zeitschriften publiziert sie Richtlinien und Zusammenfassungen über den wissenschaftlichen Stand der Kinderlähmungsfragen).

Für die Behandlung der akuten Kinderlähmung sind 79 Behandlungszentren meist in Kinderkliniken entstanden. Sie dienen vor allen Dingen der Behandlung atemgestörter Poliomyelitis-kranker und sind eingerichtet nach

den Richtlinien der Vereinigung zur Bekämpfung der Kinderlähmung.

Für den Transport in die Behandlungszentren stehen Spezialwagen des Deutschen Roten Kreuzes zur Verfügung. Diese Wagen sind mit Atemgerät und Absauggerät ausgestattet und mit geschultem Personal besetzt.

Seit dem 1. April 1957 ist ein neues Körperbehindertengesetz in Kraft. Dieses gibt auch für die Nachbehandlung und Wiedereingliederung der infolge Kinderlähmung Körperbehinderten die ärztliche wirtschaftliche und soziale Grundlage. Es sichert die orthopädische Überwachung, die Kostenregelung für Heilverfahren und orthopädische Hilfsmittel und umfasst auch die Herstellung der Erwerbsfähigkeit und Berufsförderung.

Nach Abschluss des akuten Erkrankungsstadiums und Entlassung der Erkrankten aus den behandelnden Kliniken und Krankenhäusern werden die Nachbehandlung und Nachbetreuung durch den Landesarzt gemäss den Bestimmungen und Möglichkeiten des Körperbehindertengesetzes geregelt. Dieses Nachbehandlung erfolgt in besonderen orthopädischen Kliniken und orthopädischen Kinderkliniken. Daneben stehen auch verschiedene Thermalbäder zur Verfügung, die sich speziell auf die Nachbehandlung der Kinderlähmung eingestellt haben.

### IMPFUNGEN

Zur Durchführung von Schutzimpfungen gegen Kinderlähmung wurden im April dieses Jahres 1300 l Impfstoff aus USA und 150 l aus Belgien eingeführt. Die Impfungen wurden anfangs im wesentlichen beschränkt auf Kinder des 2. und 3. Lebensjahres. Die Impfung ist freiwillig. In einigen Ländern der Bundesrepublik ist sie völlig kostenlos, in anderen trägt die öffentliche Hand nur bei wirtschaftlicher Bedürftigkeit der Eltern die gesamten Impfkosten, sonst muss der Impfstoff von den Eltern bezahlt werden. Die Impfungen werden unter Aufsicht der Gesundheitsämter durchgeführt. Die Impfbeteiligung schwankte zwischen 20% und 80% der genannten Jahrgänge. Im ganzen wurden ca. 450 000 Erstimpfungen durchgeführt. Eine Kontrolle und Auswertung der Impfergebnisse erfolgt durch das Bundesgesundheitsamt.

Ab Herbst 1957 wird voraussichtlich deutscher staatlich geprüfter Impfstoff der Behringwerke zur Verfügung stehen

# DEUTSCHEN DEMOKRATISCHEN REPUBLIK (EAST)

DR A KUKOWKA

In der Deutschen Demokratischen Republik ist die Zahl der Polioerkrankungen auch in den letzten Jahren gering gewesen und ging seit 1954 zurück

	MORBIDITÄT (AUF 100 000 E.)	LETALITÄT
1954	6.58	11.38%
1955	5.72	9.00%
1956	3.85	7.40%

Alle Poliofälle und Verdachtsfälle werden in Poliomyelitiszentren für akute Fälle deren Zahl 20 beträgt hospitalisiert und von Spezialärzten behandelt. Die Beatmungsabteilung in diesen Zentren muss mit allen notwendigen vielseitigen Apparaturen und Instrumentarien in ausreichender Anzahl sowie mit dem erforderlichen speziell ausgebildeten Personal ausgestattet sein. Nach Ablauf der vorgeschriebenen Isolierung und Behandlung werden die Kranken durchschnittlich nach 6 Wochen in die nach modernen Gesichtspunkten eingerichteten Poliomyelitis-Rehabilitationszentren verlegt. Die Zahl dieser Zentren beträgt sechs. Eines davon befindet sich in einem Thermalbad. Die Gesamtdauer der Behandlung beträgt bis zu drei Jahren und länger und zwar auf Kosten der Sozialversicherung bzw. der Staatlichen Fürsorge.

Für die Behandlung von Atemlähmungen für akute Fälle stehen in den "Poliomyelitiszentren" für akute Fälle etwa 120 Tank-Respiratoren (Eiserne Lungen), moderner Bauart sowie Apparaturen zur Durchführung der intratrachealen Beatmung gemäss der Lassen-Methode zur Verfügung. Bei spinalen Atemlähmungen wird die Verwendung von Tank-Respiratoren (Eiserne Lungen) bevorzugt. Bei bulbären Atemlähmungen ist die intratracheale Beatmung, die Methode der Wahl, während bei Schlucklähmungen Lagerungswechsel, exakte Aspirationen usw. sowie die Anwendung des selbsttätig wir-

kenden Speichelsaugschlauchs nach Denecke empfohlen wird. Es wird prinzipiell nicht allein auf die Auswahl der jeweils optimal geeigneten Apparatur sondern vor allem auf die Qualifikation des Beatmers Wert gelegt. Bei allen Beatmungsapparaturen soll grundsätzlich neben dem positiven auch der negative Druck berücksichtigt werden. Neben laufenden Kreislaufuntersuchungen usw. wird speziell auf die Kontrolle der CO-Ausatmungsluft mit einem wandfrei funktionierenden Apparat (Uras M-Firma Hartmann & Braun Frankfurt a. Main, Infrared Analyzer Beckmann und Spezial-CO-Messgerät Firma Dräger Lübeck) Wert gelegt. pH-Messungen im Blut mittels der elektrometrischen Methode können vorläufig nicht als völlig exakt anerkannt werden.

Zwecks schneller Erfassung und Betreuung von Atemlähmungsfällen sollen motorisierte Teams ausgestattet mit transportablen Beatmungsapparaturen und allem sonst notwendigen Instrumentarium zum Einsatz kommen.

Für die Behandlung der Poliofälle speziell der Atemlähmungen werden geeignete Ärzte (einschliesslich HNO-Ärzten und Anästhesisten) ausgesucht bzw. in Spezialkursen und in wiederholten Fortbildungslehrgängen herangebildet, ebenso Krankenschwestern, Pfleger und Mechaniker für die Beatmungsapparaturen.

Neben einigen kleinen Firmen widmet sich eine leistungsfähige Fabrik für medizinische Apparate der Produktion von Tank-Respiratoren (Eisernen Lungen) und von Beatmungsgeräten für intratracheale Atemlähmungen. Ein Arbeitskreis von Spezialingenieuren und medizinischen Fachleuten berät regelmässig die einschlägigen Firmen. Es wird angestrebt auf dem Gebiete der Beatmungsapparaturen möglichst einfach zu bedienende, dabei solide und gemäss dem Stande der Wissenschaft hochwertige Apparaturen herzustellen.

Ein modernes Institut für Poliovirusforschung und Immunbiologie ist eingerichtet und hat seine Arbeit begonnen.

Als beratende Organisation auf dem Gebiet der Polio-Probleme steht dem Ministerium für Gesundheitswesen das Poliomyelitis-Komitee zur Seite, dem Fachleute aus allen Disziplinen angehören. Das Poliomyelitis-Komitee steht im regen Gedankenaustausch mit den entsprechenden Organisationen vieler anderer Länder.

Polioimpfungen haben im Bereich der Deutschen Demokratischen Republik bisher nicht stattgefunden nicht etwa infolge eines grundsatzlich ablehnenden Standpunktes viel mehr deshalb um zunachst die weitere Entwicklung des Gesamtproblems abzuwarten

## GREAT BRITAIN ENGLAND AND WALES DR W H BRADLEY

I recall that at the second conference in this series Dr Christopher Andrewes mentioned certain gibes at Great Britain because her previously low incidence of poliomyelitis would place her among the less advanced countries. He added: Unfortunately we have gone up in the world since then.

In this respect our first rise to eminence was in 1947. Now in the first part of 1957 the incidence of polio was higher than ever before the order of magnitude at the 23rd week being

1957—1 082 uncorrected notifications

1950— 840 uncorrected notifications

1953— 766 uncorrected notifications

The figures which follow relate to England and Wales which is a fair sample of the experience of Great Britain. The population of England and Wales is 44 667 000 and since the last conference the corrected notification rates of acute poliomyelitis (including polioencephalitis) both paralytic and nonparalytic have been

1954— 4.4 per 100 000

1955—17.7 per 100 000

1956— 7.2 per 100 000

The deaths numbered 134 770 and 137 respectively.

The heavy incidence in 1955 was exceeded only by 1947 and 1950 our peak years. The year 1956 fell into a more normal group with the years 1951 to 1954 inclusive but the seasonal curve was unusual with a second peak in the 41st week followed by a hangover into 1957 at a considerably higher level than usual. This has been inflated further by the occurrence of 3 or 4 discrete off-season outbreaks which deserve special study and also call attention to the difficulties of forecasting the incidence of poliomyelitis.

Steady progress is being made toward meeting the ever increasing demand for diagnostic

facilities. Isolation of polio virus is now practiced in 10 of the Public Health Laboratories where in 1956 over 800 strains of polio virus were isolated equivalent to almost one third of reported cases. Eighty seven per cent of these strains were Type 1, 7 per cent were Type 2 and 6 per cent were Type 3<sup>8</sup> and investigations into the distribution of disease producing virus are now proceeding against a background of antibody studies which have given a complicated picture. For example in a remote rural district where the disease had never been reported titers by tissue-culture neutralization test were as high as in the great city of Liverpool and infection with all 3 types of virus apparently had taken place recently in both areas.<sup>9</sup>

In the large urban area of Southend 70 per cent of children under 5 had no polio antibodies but in Belfast 80 per cent of children of the same age had antibody to all 3 types of virus (Editorial 1956). It was under these circumstances at Belfast that Professor G. W. A. Dick attempted and discontinued the use of a live attenuated vaccine.

Regarding formalized vaccine at the time of the Francis report in April 1955 manufacture of a vaccine incorporating the same 3 strains used in the USA had already begun. But later in the autumn of 1955 it was decided to substitute a Brunenders Type 1 strain for the Mahoney strain previously used and early in 1956 a vaccination program for children born in the years 1947 to 1954 was announced. It was anticipated that the demand for vaccination would greatly exceed the supplies of vaccine which could be produced in the limited time available before the onset of the 1956 epidemic season and the opportunity was taken of issuing the vaccine in such a way as to allow its protective value to be scientifically assessed. There was enough vaccine only for 700 000 of the 1 900 000 children who registered for vaccination and by giving vaccine only to children born in certain designated months it was possible to accept those who registered but received no vaccine as strictly comparable controls. It so happened that the number of children vaccinated was about the same as in the placebo controlled study in America in 1954. There was not a high incidence of poliomyelitis during the observation period with the result that the number of cases were small. However, protec-

tion of the same order as was observed in the American trial has now been demonstrated and is quite substantial over the wider age range of 1½ to 9½ years. Vaccination with 2 doses administered at monthly intervals intramuscularly into the left arm is now proceeding at the rate of about 500 000 inoculations a month in the children of this age group and it is intended to continue immunization throughout the poliomyelitis season. There appears to be no doubt that the Brunenders strain incorporated in the British vaccine has conferred protection against the Type 1 infections prevalent in the country. The antigenicity of the vaccine to all 3 types was in the same range as that obtained with American vaccines.<sup>8</sup> A second manufacturer is now coming into production and a rapid improvement in the supply position can be expected.

This trial like the Francis trial revealed that the incidence of illnesses reported as nonparalytic poliomyelitis was uninfluenced by vaccination and it is instructive that whereas virus was isolated from about 70 per cent of the specimens examined in cases reported as paralytic in the nonparalytic group the virus was isolated from only 26 of the 89 specimens obtained. The pathogenesis of aseptic meningitis reported as poliomyelitis but due to other causes remains a problem and is the subject of several investigations in the United Kingdom.<sup>1</sup> In the past 3 years the ratio of nonparalytic to paralytic notifications has been 1954 32.7 per cent 1955 41.4 per cent 1956 46.3 per cent. These percentages are to some degree a measure of the frequency of disease simulating poliomyelitis and consequently the calculated fatality from polio must be interpreted with caution. Nevertheless it is gratifying to notice that mortality ratios for paralytic cases alone in 1955 and 1956 were 7.3 and 8.0 respectively showing a distinct improvement on the previous decade when the corresponding figures had been as high as 14.2 per 100 notifications. This improvement could be due to a change in the character of the disease or to more accurate observation of minor degrees of paralysis but it may also be the result of better understanding of the treatment of patients with severe respiratory disease and the establishment of many special units for this purpose.

Reports on the provoking effects of certain

inoculations first appeared in the English journals in 1950. A Committee of our Medical Research Council has reported approximate estimates of the risks associated with individual prophylactics,<sup>5</sup> the average being 1 in 3/000 inoculations in the years 1951 to 1953 and steps are being taken to control this hazard. The provoking effect of tonsillectomies recent or remote has also been confirmed in a controlled study.<sup>4</sup>

In addition to our comprehensive national health and welfare services which provide the basic needs of those stricken with poliomyelitis there are 3 voluntary bodies devoted entirely to their succor: the Infantile Paralysis Fellowship, the National Fund for Poliomyelitis Research and a co-ordinating United Kingdom Committee for Poliomyelitis established in 1956.

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## HUNGARY

### PROF. GEORGE IVANOVICS

The incidence rate of poliomyelitis has increased somewhat in Hungary recently. The morbidity rate was 12.1 in 1943 and 13.9 in 1956 per 100 000 population. Another feature of poliomyelitis endemics in this country is the change of distribution of paralytic cases by age groups during this decade. A gradual decrease from 70 per cent in 64.4 per cent of the proportion of age group 0 to 4 years occurred between 1950 and 1952. On the other hand there was an increase in the proportion of this age group since 1953 (73.2%) the trend of which reached its maximum of 81.1 per cent by 1954 and 1955.<sup>5</sup>

Poliomyelitis strains isolated from paralytic cases and typed during the last few years are shown in Table 11

TABLE 11 DISTRIBUTION OF TYPES OF POLIOMYELITIS STRAINS ISOLATED IN HUNGARY BETWEEN 1954-1956

YEAR	NO OF STRAINS	DISTRIBUTION OF STRAINS			AUTHOR
		1	2	3	
1954	10	4	5	1	Pinter <i>et al</i> <sup>a</sup>
1954	9	3	6	0	Foldes <sup>2</sup>
1955	31	6	10	15	Molnár and Fornosi <sup>4</sup>
1956	8	3	4	1	Beladi and Szollosy <sup>1</sup>
Total	58	16	25	17	

The distribution of strains by their types is rather uncommon. Type 2 strain dominated the recent epidemics in Hungary. The strains isolated during the last 3 years were recovered from patients residing all over the country.

A study has been carried out by Pinter *et al*<sup>a</sup> regarding the distribution of antibodies to different types of virus in our population (Table 12). Healthy inhabitants from the city of Szeged with no poliomyelitis history in their past were bled in 1954. Their serum samples were tested for the presence of neutralizing antibodies in cultures made of human fetal tissues.

The distribution of antibodies found in the inhabitants of Szeged in 1954 suggests that non-manifest infections with Types 2 and 3 might have been relatively rare previous to virus isolations in Hungary. The appearance and the rise of Type 2 antibody lags markedly behind Type 1 antibody. A similar tendency somewhat less pronounced is apparent in the case of Type 3 antibody. The low frequency of

antibody to Types 2 and 3 viruses in early ages might furnish a basis for elucidating the high proportion of Types 2 and 3 strains isolated recently. The neutralization test results, namely the strikingly low proportion of antibody to Types 2 and 3 in the early years of life, might involve a direct connection to the shift of incidence rate of disease toward very young ages in recent years.

This report was closed in April, therefore it does not deal with the severe outbreak which occurred quite recently in Hungary.

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## INDONESIA

### DR SOENIATNO

Before World War II poliomyelitis had been known to occur in Indonesia only very sporadically. The first case was diagnosed at Menado (Island of Sulawesi) in 1931.

It was in 1948 that multiple cases of poliomyelitis were reported from Pladju (near Palembang Island of Sumatra). These cases occurred among European children of the employees of the BPM (Dutch Oil Company). Almost at

TABLE 12 DISTRIBUTION OF NEUTRALIZING ANTIBODIES AGAINST THE 3 TYPES OF POLIOMYELITIS VIRUS IN HEALTHY PERSONS<sup>a</sup>

AGE GROUP	NO OF SUBJECTS	TYPE 1		TYPE 2		TYPE 3	
		POSITIVE	PER CENT	POSITIVE	PER CENT	POSITIVE	PER CENT
15-20 years	21	6	28	2	9	4	19
21-30 years	29	17	59	13	45	16	55
31-40 years	39	23	59	29	74	31	79
41-50 years	37	27	73	30	81	35	95

100 000 and other areas had small local epidemics. The most interesting event took place in Cork. In a population of approximately 114 000 102 cases of paralytic poliomyelitis occurred—a rate of 89 per 100 000. The epidemic began early in July and was virtually over in October at any rate so far as paralytic cases were concerned. This was the first epidemic of poliomyelitis recorded for this city. Newspaper publicity getting the upper hand of reason magnified the event to the city's great financial loss. The brunt of the epidemic in the city and the suburbs fell on infants and young children but some adult cases did occur and these were generally more severe. Some weeks after the commencement of the epidemic in the city small groupings of cases occurred in the surrounding country within a radius of about 60 miles. This spread took place in the country areas centered commercially on the city and the spread of the disease was neither as dramatic nor as rapid as in the city area. These foci began in late July and persisted into February of this year. Generally speaking the age incidence in the country areas was higher than that in the city tending to be more in the 7 to 10 year-old children and there were isolated cases in adults. In total these city and country cases accounted for somewhat less than half of the national total.

It is not easy to explain why this epidemic occurred in Cork. Cork is a port having a busy passenger traffic with the United States and there is also a frequent boat service to Britain. About 6 weeks before the epidemic an International Film Festival had been held in the city and Cork had been the center of several other national conferences. There is a pure water supply to the city but crude sewage is discharged untreated at many points straight into the river which runs through the city center. This river is highly polluted but the epidemic was unassociated with bathing. All these factors with the exception of the Film Festival have been part of Cork life since at least the end of World War II. One must presume the introduction of an invasive virus into a relatively nonimmune child population. Virus isolation was not attempted for every case but all viruses isolated were of Type 1.

Public health measures to control the spread of the infection were those advocated in the

*First Report of the W.H.O. Expert Committee on Poliomyelitis* and while it was felt that what was being done was logical the results did not seem to be impressive.

During 1956 a survey of antibody levels against poliomyelitis was carried out by the Irish Medical Research Council. Sera were collected from children on their first admission to the hospital and all the specimens had been taken before the epidemic began in the second half of the year. In addition a number of adult sera were collected at blood transfusion donor clinics. These specimens were representative of the different areas in the country and the numbers in each age group were as follows:

0-4 years	86
5-9 years	159
10-16 years	125
18-30 years	100

The final report is not yet available but taking the country as a whole the results in the 5 to 9 year group show 43 per cent of children with antibodies to Type 1 51 per cent to Type 2 and 62 per cent to Type 3. The results for Type 3 are somewhat unexpected—only a small number of strains of this type have so far been isolated in Ireland. As a result of the experience during the second half of 1956 the level of Type 1 antibody may now be somewhat higher as nearly 80 per cent of the poliomyelitis viruses isolated during that period proved to be Type 1 and it is clear that this was the type responsible for the 1956 epidemic.

At the beginning of 1956 our 3 main acute poliomyelitis treatment centers had been staffed and equipped. Of the 499 paralytic cases the great majority were treated at one or other of these centers about 13 per cent requiring treatment for breathing or swallowing difficulties. One of our major problems in the basing of our treatment on these 3 centers is the large area each center must serve. It is difficult to obtain agreement on the best methods of transporting acutely ill cases to the centers. We have constructed a large ambulance which carries an electrically operated tank respirator and has facilities for intermittent positive pressure and suction. This ambulance is staffed by an anesthetist, a physician and a nurse. It is situated at Dublin, the center serving by far the largest district. The other 2 centers rely on their ordinary ambulances which are staffed with similar

teams and use one or other form of manually assisted respiration if required to bring their cases from their homes to the center

Twenty deaths attributable to poliomyelitis occurred during 1956 giving a rate of 4 deaths per 100 paralytic cases. This compares with the rate of 17 per 100 paralytic cases in previous years before the regionalization of treatment. It is very doubtful if all deaths due to polio are certified accordingly. Some deaths shown on postmortem to have been caused by polio have baffled even the most astute clinicians and had been thought to be due to another cause.

Since 1947 we have had a growing pool of persons crippled by this disease. The Minister for Health has set up the National Organization for Rehabilitation to advise him on the various problems involved. So far this work is in its infancy.

In May 1957 vaccination against poliomyelitis was introduced for the first time using a British manufactured vaccine. For a number of reasons the vaccine has been offered to the 1 and 2 year-olds initially. The course consists of 3 1 ml injections given intramuscularly the first 2 at about a 4 week interval and the third at least 7 months later. This vaccination scheme was offered free of charge to those in the eligible groups and of 125 000 infants in these groups the parents of 11 000 have applied for vaccination. We have now extended the scheme to children under 5 years.

## ISRAEL

### DR P YEKAUTIEL

The transition of poliomyelitis from a sporadic to an epidemic disease began in Israel in 1950 with a formidable epidemic of 1 600 cases representing an attack rate of 146 per 100 000. Ever since the annual incidence has never been below 30 per 100 000 with an average of 47 for the last 5 years—one of the highest rates in the world.

The age distribution of our cases of infantile paralysis is similar to that in other countries in the Middle East. The highest age specific rates are found in the first and the second year of life and almost 80 per cent of all cases are in children under the age of 3. This age distribution has not changed since 1950.

The hospitalization and the rehabilitation of

poliomyelitis cases is a major burden for the country. All cases are hospitalized free by the government. A large rehabilitation center has been developed in one of our government hospitals.

In view of this serious poliomyelitis problem the Ministry of Health began the planning of mass vaccination as soon as the encouraging results of the Salk vaccine trials in the USA were published in April 1955. As it soon became clear that supplies of vaccine from abroad could not be relied on for an uncertain period these plans included the first steps for the local production of a Salk type vaccine in Israel. Preliminary tissue-culture work was carried out in collaboration by several virus laboratories. In January 1956 the Ministry of Health's special poliomyelitis vaccine laboratory was opened. Actual production of vaccine did not start until April of the same year since only then after many delays and difficulties did we receive the first shipment of monkeys. Yet in January of this year the first lot of trivalent formalized vaccine tested for safety and potency both in Israel and abroad was used in our vaccination campaign.

Our first antipoliomyelitis vaccination program comprised infants and children from the age of 6 months to 3 years. Two basic inoculations were given during the winter period January to April of this year with the booster injections scheduled for this autumn. The response of the public to the voluntary program was beyond our expectations of the 123 000 children in the eligible age group 115 000 i.e. more than 90 per cent received their first 2 injections. This is the more remarkable since we were not dealing with school children who are regimented much more easily for such vaccination programmes.

In order to save vaccine and relying on the experience in Denmark we gave intradermal injections of 0.3 cc to a large proportion of the children and 0.5 cc subcutaneously to the others. In addition to the local vaccine an equal amount of Salk vaccine from the USA released by the U.S. Public Health Service was used in the program.

It is of course too early yet for a definite evaluation of the results of this vaccination in Israel. However the following data are of interest. Our poliomyelitis season starts early



reaching its peak between May and July. The average number of cases during the February to June period over the last 5 years was 270; the minimum number was 106. This year the total number of cases over the same period was 13, of which 8 had not been vaccinated. The total number of poliomyelitis cases in vaccinated children aged 6 months to 3 years during these 5 months was 5, representing an attack rate of 0.43. The attack rate in nonvaccinated children of the same age group was 4.3.

The Israel Government is grateful to virologists of the USA and Denmark without whose valuable help and advice the local production of vaccine could not have succeeded in such a short time.

## ITALY

PROF GIUSEPPE PENSO

La poliomyélite continue à être en Italie une maladie de la première enfance. La plupart des cas déclarés sont en effet des enfants âgés de moins de 4 ans. Le nombre de cas déclarés s'élève par année à environ 7 700 sur une population de 50 000 000 environ d'habitants. Malgré le nombre limité de cas, le virus polio est très répandu chez les enfants sains. D'après les recherches faites à l'Institut Supérieur de la Santé de Rome, il résulte que dans les communautés de nombreux enfants sont porteurs de virus polio virulents. Dans certaines communautés exemptes de poliomyélite on trouve jusqu'à 75 % de porteurs de virus poliomyelitiques virulents.

En Italie on a eu plusieurs épidémies de méningo-encéphalite dues à différents types de Coxsackies, entre autres à un virus Coxsackie A, dont le typage a été impossible avec les 19 types d'antisérum A. D'autres cas de méningo-encéphalite on a isolé des virus cytopathogènes dont le typage a été impossible.

Cette année on a commencé en Italie la vaccination contre la polio avec un vaccin étudié et préparé en Italie et contrôlé par l'Institut Supérieur de la Santé. La réponse immunitaire à ce vaccin a été très bonne.

La vaccination contre la poliomyélite n'est pas obligatoire en Italie et elle est pratiquée gratuitement par l'Etat aux enfants qui vivent en communautés. On vaccine seulement au-dessous de 4 ans.

## JAPAN

DR MASAMI KITAOKA

At the last Conference the outline of polio in Japan was announced and it was mentioned that a few Japanese investigators had started studies on the disease in laboratories expecting fruitful results in the future. Since then information has been received concerning the isolation of the virus from various samples, the identification of new isolates, the immune state of inhabitants on sera collected from certain age groups in different areas all over Japan and also the studies on tissue culture carried out on the basis of cytopathogenic effect of the polio virus on the growing cells at several laboratories, for example in Sapporo (Dr Hanamitsu) in Tokyo (Drs Matsumoto, Takatsu, Kono and Nakaki) in Matsumoto (Dr Yamada) in Osaka (Drs Nishizawa and Tatsumi) and in Kumamoto (Dr Rokutanda). Our institute has been functioning as the WHO Poliomyelitis Regional Center in the northwest Pacific area since September 1955, not only for the epidemiologic survey in the field but also for the laboratory work.

As for the incidence of polio in Japan a few thousand cases per year, paralytic and non-paralytic, with a fatal case rate of approximately 20 per cent were reported according to the Welfare Ministry since 1948 when the disease was classified as being notifiable in Japan. The number of reported cases reached the maximum of 4,233 (4.7 rate per 100 000 inhabitants) in 1951 and then decreased gradually in number year by year following neither vaccination nor special preventive procedures. In 1955 and 1956 the number of cases reported was 1,314 and 1,497 respectively (roughly one third of those in 1951) although 2 epidemics, one in Sapporo, Hokkaido Prefecture and the other in Shusho, Ehime Prefecture, occurred in the last year. All new isolations from the cases in both epidemics were identified as Type 1 polio viruses. Japan is still strongly endemic of polio virus as already pointed out by Dr Paul about 10 years ago because almost all cases were in children under 4 years old. The morbidity rate in Shusho (0.69 %) was twice as high as that in Sapporo among the age group under 4 years and it is surprising that 1-year-old children suffered from polio paralytic and nonparalytic by

the ratio of 1 108 per cent in Shuso. The ratio of paralytic to nonparalytic polio cases was 3 1 in Sapporo and vice versa in Shu. This might be due to different sampling in both areas that is cases in Sapporo were reported by local physicians while those in Shuso were found by checking personally each inhabitant by a case finding team.

In the laboratory virus isolation has been undertaken continuously from the patients in Aomori Tokyo and Osaka since 1954. As a result out of 134 samples (stools) 67 (50%) polio viruses were found. These consisted of 36 Type 1 (53.7%) 20 Type 2 (29.8%) and 11 Type 3 (16.4%). Four nonpolio viruses (3%) were isolated. However the Type 2 virus was encountered more often in the stools from the patients (not in epidemic cases) in Aomori and Tokyo especially in Osaka in 1957 contrary to results in Osaka in 1954 and 1956. It is interesting to note that in Shuso Type 1 viruses were isolated by 40 per cent (22/55) from patients by 59 per cent (30/513) from all inhabitants of any age in epidemic areas and by 9.7 per cent (4/41) from children under 7 years old living in nonepidemic areas near Shuso. When compared with positive isolation rates in each age group among children under 7 years old of three groups (patients children in epidemic areas and children in nonepidemic areas) the children under 5 years old gave little less positive rate (about 30%) in epidemic area and much less (about 10%) in nonepidemic areas than the patients (about 45%) while in the age groups of more than 7 years old such differences among 3 positive rates were not so marked.

On the other hand virus isolation was attempted from the stools collected from healthy children in Aomori Yamagata and Tokyo. Six Type 2 polio viruses were isolated from 55 infants under 1 year old at the Setagaya Infant Home in Tokyo. The youngest virus harboring infant was 2 months and 79 days old.

During the virus isolation experiments viruses giving a cytopathogenic effect on the culture cells but not neutralized by the immune serum of any type of polio virus were encountered. The cytopathogenic effect of such new isolations on human embryo cells HeLa cells monkey kidney cells and human amnion cells

as well as the results of identification are tabulated though those experiments are not yet all completed. Out of 16 strains 5 were identified as adenoviruses (Type 1 1 Type 2 3 Type 5 1) 1 as a Coxsackie virus and 6 as ECHO viruses (both latter viruses not yet typed). The remaining 4 are still unknown.

The immune state of inhabitants was surveyed against polio virus (Drs Takatsu Yamada and Kono) and against Coxsackie virus (Drs Fukumi and Hamagami). Furthermore the usefulness of complement fixation test on polio the practical use of color change (pH change) of culture media and the plaque forming on monolayer of cells after inoculation of polio virus the mutant polio virus resistant to the inhibitor in bovine serum HeLa cell adapted ECHO virus the plaque forming of ECHO virus on monolayer of HeLa cells and ECHO virus resistant HeLa cells were investigated.

As for vaccine a killed vaccine (like Salk's vaccine) has not yet been prepared in Japan. However the immune response both complement fixing and neutralizing antibodies was checked in Japanese infants from 6 months to 4 years old (mostly 1 to 2 years old) following 3 shots of a 1 ml subcutaneous dose or an 0.1 ml intradermal dose the first 2 shots given 7 days apart and the third shot as a booster given 6 months later using the vaccine made in the U.S.A. The placebo vaccine was prepared in my laboratory from tissue culture without virus inoculation. The results so far obtained were almost similar to those reported by Dr Francis that is the poor response against Type 3 virus. The avirulent strain of Type 2 polio virus was reported at the last conference but such strains had not proved to be avirulent after intraspinal inoculation. After all any completely avirulent strain has not yet been obtained in Japan.

## KENYA

DR J. H. HARRIES

The first large epidemic occurred in Kenya in 1953-1954. Approximately 700 paralytic cases were notified during that year. The incidence among the various racial groups is interesting.

Europeans	2.6 per 100 000
Asians	6.0 per 100 000
Africans	12 per 100 000

Since December 1956 we have been experiencing a further epidemic. So far 400 paralytic cases have been notified. During the past 3 weeks 60 cases have been notified. In Nairobi about 30 cases a month have been admitted since the beginning of the year.

The majority of cases occur in the towns starting at the coast in Mombasa spreading to Nairobi then proceeding up country.

Only Type 1 virus has been isolated from any of our paralytic cases.

In 1954 a respiratory unit was set up in Nairobi. This unit accepts cases from all over Kenya, Uganda and Tanganyika. As the roads in Kenya are bad and large distances have to be covered an air rescue service has been set up to transport respiratory cases. Approximately 20 flights have been made.

During this year we have admitted 45 cases of respiratory paralysis; there have been 7 deaths. Ten per cent of these cases had bulbar paralysis, 20 per cent had bulbospinal paralysis and 70 per cent had diaphragmatic or intercostal paralysis.

Vaccination against poliomyelitis was started this year using the Salk vaccine. So far 4 000 children have been vaccinated but we are experiencing difficulty in obtaining adequate supplies of vaccine.

## LEBANON

DR JOSEPH HATEM

Avant 1939 la poliomyélite était très rare au Liban, presque inexistante. C'est à partir de cette date que l'attention des responsables fut attirée par l'apparition de quelques cas localisés dans la région du sud du pays aux confins de la Palestine. Jusqu'en 1951 l'indice de morbidité pour 100 000 habitants ne dépassait pas 0.40.

En 1952 le nombre de cas déclarés passe brusquement à 58, soit un indice de morbidité de 4.6 et après des variations atteint en 1956 le chiffre record de 82 cas, soit un indice de 6.5. Nous ne savons pas exactement la raison de ce changement brusque et de cette extension de la maladie; il coïncide toutefois avec l'arrivée dans la capitale d'un grand nombre de ressortissants étrangers; d'ailleurs les chiffres cités plus haut comprennent les cas survenus chez ces étrangers en résidence temporaire dans le pays.

Il est à remarquer que les chiffres cités plus

haut sont inférieurs aux chiffres réels car l'ordre de déclaration de la poliomyélite n'est pas toujours respecté. De plus tous les diagnostics concernant les cas rapportés sont des diagnostics cliniques.

Quant à la fréquence saisonnière on peut constater qu'il existe une morbidité maximale en 1953 au printemps et en été en 1954 en été et en automne en 1955 au printemps jusqu'à l'hiver en 1956 principalement en été.

En tenant compte des réalités climatiques nous pouvons donc déduire que la poliomyélite au Liban a une prédilection pour la période chaude et sèche, période de 6 mois environ qui va de mai à octobre avec un maximum en juin, juillet et août, c'est-à-dire aux mois qui correspondent à l'ère classique.

Nous regrettons de ne pas disposer de chiffres statistiques valables sur la mortalité; la répartition suivant l'âge et la gravité des paralysies.

En outre il existe des cas sporadiques durant les mois froids et humides de novembre à avril.

Il résulte que depuis 1952 l'augmentation relativement considérable de la morbidité de la poliomyélite pose un problème nouveau au Liban.

Dans la lutte antipoliomyélique que nous devons mener désormais, quels sont les moyens dont nous disposons?

Du point de vue hospitalier, il faut reconnaître que nous manquons de facilités et de personnel spécialisé pour soigner les maladies graves à la phase aiguë. Nous ne disposons que de quelques poumons d'acier et si par malheur une grave épidémie se produisait nous serions à court d'appareils et de personnel spécialisé.

Par contre en ce qui concerne les séquelles et la réadaptation fonctionnelle un centre de rééducation fonctionne déjà à Beyrouth. Il a été créé grâce au concours de l'Abbé A. Corbiac de la Cite des Apprentis, l'Union libanaise de la Protection de l'Enfance, les deux facultés de Médecine de Beyrouth, le Ministère de la Santé, l'UNICEF et l'OMS. Ce centre est un modèle du genre. Il est bien équipé et doté d'un personnel qualifié. D'autres centres de ce genre sont en voie d'achèvement. En outre trois hôpitaux privés de Beyrouth ont déjà leur département pour la réadaptation des infirmes-moteurs.

Enfin la mise en marche imminente du Laboratoire National d'Hygiène Publique nous per-

mettra dans un proche avenir de pouvoir isoler des souches et de déterminer les types predominants responsables de l'infection et surtout de mener une enquête serologique afin de déterminer la protection de la population suivant les âges et décider de l'éventuelle nécessité d'une vaccination antipoliomyélique appliquée aux groupes d'âge les plus exposés

## NETHERLANDS

Dr H J DYKHUIS

In 1956 more than 2 700 cases of poliomyelitis were registered in the Netherlands. This means an incidence of 20.4 per 100 000 inhabitants.

The year 1943 follows immediately with 1,931 cases but the morbidity per 100 000 inhabitants was higher to wit 21.3.

In 1956 epidemic outbreaks of virus meningitis (caused by the ECHO virus) were also reported all over the country. This certainly interfered with the diagnosis of nonparalytic poliomyelitis.

Nevertheless in 1956 proportionally no larger number of nonparalytic cases has been notified. A 4 times larger number of paralytic cases is usual in the Netherlands. Last year this was also the case.

With regard to the distribution according to sex it can be mentioned that a larger number of cases was reported among men than among women.

As to age the largest number of paralytic cases was recorded among the youngest age groups to wit 60.7 per cent from 0 to 4 years of age and 74.9 per cent in the group from 5 to 9 years of age. This age pattern is rather constant in our country. A shift to a higher age group did not occur.

The first symptoms of an epidemic occurred in the 71st week and the peak was reached in the period from the middle of July to the middle of August. A year with a much higher morbidity than average preceded 1956, a fact that was also noted in earlier epidemics. This occurrence made it possible for us early in 1956 to be alerted to the possibility of an outbreak of an epidemic so that measures were taken in order to be able to fight its conse-

quences. The predominant idea was that modern treatment of poliomyelitis required that care should be given in special centers where the best care is available because teamwork is possible and modern apparatuses are used. This applies especially to patients with respiratory distress.

With the aid of the Government 9 polio centers were set up which provide mechanically functioning respirators from a pool. In this way interchange or completion of respirators was possible. In these centers a total of 230 patients with respiratory paralysis were treated i.e. about 10 per cent of the total number of cases registered. The mortality of the patients treated with respirators was no more than 20 per cent. (Up to now 50 patients are being treated totally or partially with respirators. 18 of whom probably will be in permanent respiratory distress.)

Besides these centers a number of rehabilitation clinics were rapidly established where individual physical-therapeutic treatment in small units was especially accentuated. At the same time possibilities for ambulatory treatment were created.

In 1956 the central authorities, the sick funds and the national private organization *Het Prinses Beatrix Poliofonds* gave financial support in order to fight poliomyelitis. On a voluntary basis the population collected large sums of money on behalf of the fund.

With regard to vaccination against poliomyelitis it can be mentioned that in the autumn of this year a vaccination program will be started which will be directed and organized by the central authorities. At first this program will be limited to one age group. Next spring the number of children to be vaccinated will be increased. Vaccination will not be compulsory. In the meantime some municipalities and industries and also a certain number of private practitioners have started vaccination at their own initiative. The Government buys vaccine in the USA. Also Belgian vaccine is being imported. In the State Laboratory and in collaboration with the Netherlands Institute for Preventive Medicine a pilot study is being made with regard to the production of a Dutch vaccine.

Up to this moment only a limited number of cases of poliomyelitis have occurred in 1957.

## NORWAY

### DR PETER M HOLST

Since the last Conference in 1954 Norway has had comparatively few cases of polio. In 1954 the number of paralytic cases was 434 in 1955 191 and in 1956 only 73 (Table 16). Until now we have had only a couple of cases this year.

Vaccination was started November 1 1956 using American and Danish vaccines. The vaccination was offered to all school children in their first 7 years on a purely voluntary basis. Up to this time it is officially reckoned that about 90 per cent of 450 000 children belonging to these age groups have been completely vaccinated. After January 1 any person may be vaccinated by his private physician and we now reckon that an additional 400 000 persons are partially vaccinated. In many cases municipal authorities or industrial concerns have paid for the vaccination. From September 1 the State will offer free vaccination to all children in the age group of 9 months to 2 years. The Danish vaccine has been administered intracutaneously in the way described by my colleague Dr Juel Henningsen. The American vaccine has been applied subcutaneously. From different parts of the country about 5 000 blood samples have been collected in order to test the antigenic response of the vaccines. These samples have been taken before vaccination and after the second and the third vaccination. Until now only very few of them have been examined but results show a good response. It is too early to predict the effect of the vaccination on the epidemiology of the disease. Complications have not been registered.

The facilities for virus and serologic research have been very limited in Norway but conditions in this respect will improve considerably in the course of this and next year. So far we have no plans for producing vaccines.

Thanks mainly to our National Foundation against Poliomyelitis conditions of treatment and aftercare have been much improved during the last years. We have now 10 clinics for the treatment of poliomyelitis. This year the Foundation has opened a central clinic in Oslo which will have facilities for orthopedic surgery and neurologic and physical therapy. The total number of beds which are reserved exclusively

TABLE 16 NUMBER OF CASES OF POLIOMYELITIS WITH OR WITHOUT PARALYSIS IN NORWAY FROM 1950 TO 1957

YEAR	PARALYTIC POLIO	NONPARALYTIC POLIO
1950	706	199
1951	1 563	670
1952	527	197
1953	928	179
1954	434	150
1955	191	58
1956	73	24
1957	6	1

for the treatment of polio cases is now 300. Qualitatively but not quantitatively we have a very difficult problem which I suppose we share with other nations. What shall we do with our young respiratory cases? We have 25 to 30 young patients who are more or less dependent on artificial respiration. We feel that it would be highly unsatisfactory to bring these patients home even if it is technically possible because in their homes they will be completely isolated and they will have practically no chance to develop their minds. These patients will never be able to support themselves by manual work but most of them are intelligent. We feel that we must give them something to live for. But how? Any suggestion will be received with thanks.

## PHILIPPINE ISLANDS

### DR BENJAMIN V TANESIS

The consensus in the Philippines today by workers in public health is that poliomyelitis is not a public health problem. This opinion has probably been distilled from the following observations:

- 1 The absence of epidemics in the Philippines that would approximate the extensiveness of poliomyelitis epidemics in temperate zones.
- 2 The belief that the virus of poliomyelitis is endemic in the Philippines.

- 3 The further assumption that the population as a whole has acquired a certain degree of immunity because of its continued contact with poliomyelitis virus.

While this attitude would tend to mitigate almost any active public health measure against it, there are certain basic facts which

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TABLE 17 POLIOMYELITIS CASES AND DEATHS PER 100 000 POPULATION IN MANILA 1939-1955

YEAR	NO	CASES		DEATHS	RATE
		NO	RATE		
1939	26		4.20		
1940	63		9.56		
1941	3		0.43		
1942	16		2.17	5	0.76
1943	21		2.70	1	0.14
1944	4		0.49	4	0.54
1945	8		0.91	3	0.39
1946	5		0.59	2	0.24
1947	16		1.71	3	0.35
1948	74		1.44	9	0.33
1949	5		0.49	7	0.96
1950	176		11.97	3	0.30
1951	29		2.65	16	1.52
1952	59		5.21	5	0.46
1953	42		3.59	9	0.80
1954	156		14.89	9	0.77
1955	102		8.16	78	7.31
Total	695			16	1.78

TABLE 18 AGE INCIDENCE OF POLIOMYELITIS 1950-1954 (CASES FROM SAN LAZARO HOSPITAL)

	NO OF CASES	PERCENTAGE
Below 1 year	179	19.48
1 to 4	564	61.37
5 to 9	50	5.44
10 to 14	35	3.90
15 to 19	28	3.04
20 and above	63	6.86
Total	919	100

Therefore we are forced to the conclusion that the consensus as obtains in my country today may be wrong in the sense that there are more victims with paralysis than are reported in official statistics.

If the age group that bears the brunt of the disease is the one composed of the children from 1 to 5 years of age, this very same reason would indicate that preventive measures should be undertaken by our government to prevent or at least diminish the incidence of crippling or this disease leaves in the 1 to 5 year-old age group.

The third problem that comes up is what to do for those who have already developed the paralysis and the deformities from polio. The National Orthopedic Hospital is almost alone in its attempt at providing physical medicine modalities for the management of postpolio paralysis and in its pioneering in the recon-structive procedures that are usually available to the victims of polio paralysis in more ad-vanced countries. This effort however is in its infancy and will require considerable atten-tion so that the lessons that our orthopedic sur-geons have learned may be channeled correctly and or implemented by the developments in this type of work in the more experienced countries.

There is at present a civic organization in our country, the Philippine Foundation for Polio headed by General Basilio J. Valdez which has attempted to carry on polio work. However its efforts have been restricted because its funds are limited and it has concentrated its work mostly on direct material and in the form of hospital materials, equipment and by becomes evident that we have only a

present themselves as far as poliomyelitis is con-cerned in the Philippines.

1 There is no accuracy as is true in some areas in the reporting of poliomyelitis cases with the consequence that any available data during the time of sporadic outbreaks are data available only as far as cases that come to gov-ernment hospitals are concerned. It is safe to assume that for every case that comes to a government hospital for treatment there are many other cases unreported. The conclusion is unavoidable that whatever statistical data the country has today is in all probability inaccurate and does not present a true picture of the dis-ease (Table 17).

2 The available statistical data would seem to indicate that the ravages of polio hurt the age group between 1 to 5 years the most and that the incidence of the disease diminishes with the age of the individual (Table 18).

3 From the available records of the National Orthopedic Hospital it would seem to appear that a great amount of the orthopedic work involves giving attention to postpolio patients with paralysis.

4 There are hardly any technical personnel with extensive and sufficient training in the task of rehabilitating the polio paralytic in our coun-try.

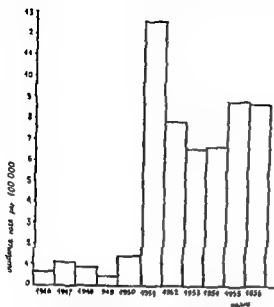


FIG 26 Polio incidence rate per 100 000 in Poland 1946-1956

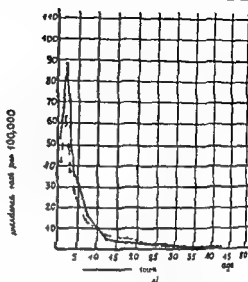


FIG 27 Distribution of poliomyelitis in Poland by age in rural and urban areas 1951-1953

surface of the entire problem but we are hopeful that in the increasing consciousness of our people for the physically disabled the civic organization in our country devoted to polio eventually will become stronger and more active in its function.

And this is the thought I should like to leave. While my government is faced with the more acute problems of tuberculosis, jaundice, malaria and schistosomiasis and while the attention of poliomyelitis workers is presently occupied for the most part by virus studies and efforts at providing a more effective prophylaxis, our main problem in the Philippines today is the provision of sufficiently trained personnel for distribution to the rest of the country so that the deformities left behind by our small sporadic epidemics—old and recent—can be attended effectively—in other words the total physical rehabilitation of the paralytic polio patient.

In closing on behalf of the Philippine Government I wish to thank the Swiss Government and the International Society for Poliomyelitis for the opportunity to be present at this conference.

#### POLAND

PROF DR F PRZESMYCKI

The incidence of polio in Poland during the years 1926 to 1951 was rather low and did not

exceed the figure of 1.5 per 100 000. But in 1951 an epidemic broke out which reached the rate of 12.7 per 100 000. During the next years the variation in the rate was rather insignificant, and the number of cases ranged from 6.6 to 8.9 per 100 000 (Fig. 26).

In the epidemic year the number of cases was 3 184 and the corresponding figure for 1956 was 2 418. The average lethality for the years 1951 to 1954 was 6.3 per cent. The number of cases in males was higher than in females. From 1951 to 1956 the cases in males rose to 55.2 per cent and those in females to 44.8 per cent of patients.

The analysis of cases by age groups shows that the highest incidence rate was seen in the age group 0 to 9 years (about 90 % of all notified cases). The distribution of the cases in percentage by age groups in 1956 was as follows:

0 to 4	78.6%
5 to 9	12.8%
10 to 14	2.8%
15 to 19	2.1%
Over 20 years	3.6%
Age not stated	0.1%

The percentage distribution of cases in 1956 is similar to that registered during the years 1951 to 1955.

The incidence of polio is higher in urban than in rural districts (Fig 27)

To the cases notified in 1951 to 1956 the following clinical classifications could be applied

Paralytic cases	90.5%
Nonparalytic cases	6.7%
Abortive	2.8%

The percentage of paralytic cases is higher in Poland than in other countries. However it should be noted that nonparalytic cases or cases with slight manifestations of paralysis often are not reported and this is probably responsible for the divergency between our data and those of foreign countries.

The investigations made in 1951 show that the epidemic was due to a Type 1 virus. Four strains were isolated from feces on monkeys. They belonged to Type 1. In 1956 there were isolated 131 strains on tissue cultures. The investigations were carried out in Warsaw and in some other towns. The distribution of isolated strains by types is given below.

Type 1	82	66.6%
Type 2	31	25.2%
Type 3	10	8.0%
Orphan strains	8	

Recently the production of vaccine by the Salk method was inaugurated. The first series of this vaccine have already been prepared. It is intended to proceed to an experimental vaccination of 100 000 persons during the current year.

## RUMANIA

### DR C. IONESCO-MIHAIESTI

Dans l'évolution de la poliomyélite en Roumanie on peut considérer 2 périodes distinctes au point de vue épidémiologique: une première, de 1927 à 1949 et une deuxième période de 1949 à 1957.

La première période commence avec l'épidémie de 1927-1928 première grande épidémie enregistrée dans notre pays (plus de 2 200 cas paralytiques)—une morbidité de 11,3 cas pour 100 000 habitants.

Pendant les 21 années non-épidémiques qui suivirent depuis 1928 à 1949 les statistiques officielles indiquent une morbidité qui n'a jamais dépassé 2,6 pour 100 000 et oscillant en général entre 0,2 à 1,5 pour 100 000 habitants.

La dernière période de 1949 à 1957 (1 janvier) est caractérisée par un rapprochement impressionnant des années épidémiques—d'une

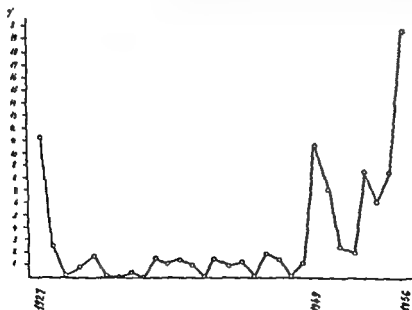


FIG. 28. Évolution de la poliomyélite depuis 1927 en Roumanie (taux par 100 000 individus)



part et d'autre part par l'installation d'un état endémique avec une incidence générale de la poliomyélite relativement élevée sur lequel apparaissent des crochets épidémiques assez importants.

Après 1949 année quand survint le deuxième grande épidémie de poliomyélite avec une morbidité de 105 pour 100 000 habitants les années épidémiques se succèdent de près 1950 avec 72 pour 100 000 1953 avec 8 pour 100 000 1955 avec 84 pour 100 000 habitants, situation qui en 1956 atteint l'incidence considérable de 187 pour 100 000 habitants (plus de 3 200 cas confirmés)—ce qui représente la morbidité par poliomyélite la plus élevée consignée jusqu'à présent en Roumanie (Graphique 28).

L'extension de la poliomyélite dans notre pays à cette incidence si élevée de la morbidité pendant la période de 1949 à 1957 s'est produite à la faveur de foyers épidémiques délimités qui n'ont jamais intéressé plus d'approximativement un tiers de la surface totale du territoire du pays.

Malgré leur étendue relativement réduite ils présentaient toutefois une incidence de la morbidité très élevée.

C'est dans ces conditions d'endémie épidémique qu'a éclaté l'épidémie de 1956 qui marque probablement une nouvelle étape dans l'évolution de la poliomyélite dans notre pays.

Considérée dans son ensemble l'épidémie de

1956 tout en gardant certains aspects épidémiologiques de la période 1949-1956 présente un envahissement massif de certaines régions relativement bien délimitées dont 2 dans la zone centrale du pays 1 dans le nord-ouest 1 autre dans le sud-ouest et une cinquième dans le nord-est.

Le total des cas enregistrés dans ces 5 régions représente 60 % du nombre total des cas apparus en 1956 tandis que la population de ces régions ne représente que 27 % de toute la population du pays. La morbidité dans ces régions a varié entre 32 à 54 pour 100 000 habitants et dans certains districts a dépassé 100 pour 100 000 habitants. Il faut ajouter que ces régions sont très industrialisées pourvues d'un important réseau de voies de communications et caractérisées par une circulation très active de la population.

L'analyse de la morbidité générale étudiée séparément pour les villes (21,2 %) et les villages (17,4 %) nous montre que l'incidence dans le milieu rural est très élevée du nombre total des cas de poliomyélite 35 % proviennent des villes et 65 % du milieu rural. L'extension dans le milieu rural pendant l'épidémie de 1956 a dans la plupart des cas pris un caractère explosif comparable à celui des épidémies de rougeole ou aux infections d'origine hydrique.

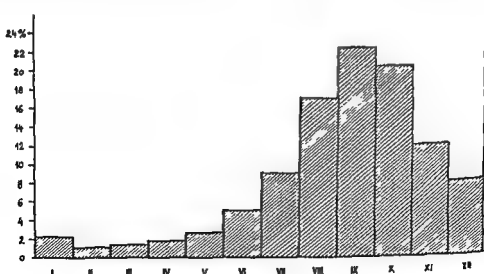


FIG. 29 Répartition saisonnière des cas de poliomyélite en 1956 (pourcentage par mois de nombre total de cas)

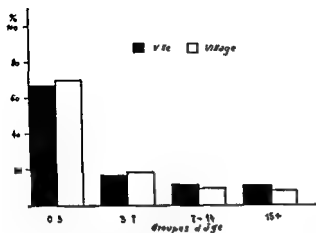


FIG 30 Répartition par groupes d'âge des cas de poliomyélite de claires dans les villes et dans les villages

En ce qui concerne le caractère saisonnier on constate comparativement aux années épidémiques antérieures un déplacement de l'incidence maxima dans les mois de septembre et octobre (Graphiques 29 30 et 31)

Un autre caractère que nous voudrions signaler est la fréquence des cas de poliomyélite parmi les enfants d'au-dessous de 6 mois (96 cas) 59 parmi ces enfants présentaient des formes paralytiques spinales 6 paralysies des nerfs crâniens 11 formes respiratoires et 70 seulement des formes nonparalytiques

Nous n'insisterons pas sur les formes cliniques et leur répartition par groupes d'âge

Le nombre de décès en général pour 100 malades a été 74

RÉPARTIS PAR GROUPE D'ÂGE	TOTAL DÉCÈS	DÉCÈS POUR 100 CAS PAR GROUPE D'ÂGE
0-3	69%	77
3-7	15%	68
7-14	8%	60
15 et plus	8%	91

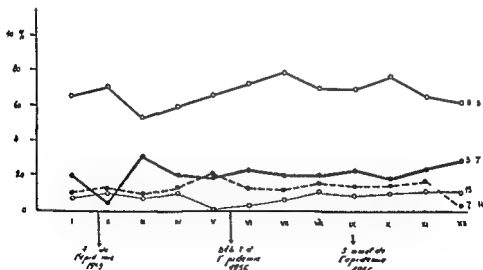


FIG 31 Répartition saisonnière des cas de poliomyélite par âge (pourcentage par groupes d'âge du nombre de cas par mois)

La proportion de decés pour 100 malades montre la gravité de l'épidémie de 1956. La fréquence élevée de ces décès en milieu urbain comparativement au milieu rural tient en grande partie au nombre plus élevé des malades de plus de 15 ans en milieu urbain.

Avant de terminer ce court exposé je voudrais mentionner quelques résultats de nos examens, effectués à l'Institut Dr J. Cantacuzène sur plus de 550 fèces provenant de malades ou de leur entourage pour la mise en évidence du virus et la détermination des types.

Nous avons isolé 114 souches dont

110 Type 1	(96.49%)
1 Type 2	(0.87%)
3 Type 3	(2.64%)

Nous n'avons jamais rencontré la coexistence avec les virus Coxsackie.

Je me permets en terminant ce rapport de féliciter et remercier au nom de notre Gouvernement l'Association Nationale pour la Poliomyélite des Etats Unis d'Amérique pour l'organisation de cette quatrième Conférence et d'exprimer la gratitude de notre pays pour l'hospitalité qui nous a été offerte par la Suisse.

## SWEDEN

DR GUNNAR OLIN

A severe outbreak of polio occurred in Sweden in 1953 with 47 paralytic cases per 100 000 during the epidemic year from April 1953 through March 1954. Since then the polio incidence has been low and slowly decreasing. In 1954 there were only 5 cases per 100 000, in 1955 4 cases, in 1956 3.5 cases per 100 000.

The big outbreak of 1953 offered extensive opportunities for clinical studies of the disease and for investigations concerning its treatment. The Stockholm hospital for epidemic diseases has made an important contribution in this field.

Much interest has also been devoted to the problem of vaccination against polio by a team in Stockholm. A Swedish polio vaccine produced from virus cultivated on human embryonic tissue was tried on 2 000 children early in 1955. No untoward reactions were observed. However, the Cutter incident as well as findings in Swedish control tests necessitated improvement of the safety of the vaccine. It has

also been considered important to try to increase the immunizing capacity of the vaccine. Owing to the work connected with the solution of these problems large scale production has been retarded.

Therefore the first immunization program had to be carried out mainly with vaccine from the USA released by courtesy of the federal authorities. When the immunizations started in February of this year only about 100 liters of Swedish vaccine produced from virus cultivated on monkey tissue were available. The program included children aged 4 to 11 years. About 900 000 children were offered vaccination free of charge, about 700 000 accepted and by now have received 2 inoculations each of 1 ml 3 weeks apart. The program has been accomplished without an accident.

The immunizing capacity of the vaccine used has been titrated both in children and in guinea pigs. The result is presented at the Scientific Exhibit of the Conference.

Since more Swedish vaccine became available in May it has been possible to extend the vaccination to certain categories at risk, namely the staffs of virus laboratories and hospitals for epidemic diseases, pregnant women and on a smaller scale people aged below 35, at their own expense. The conscripts on duty in the armed forces have also been vaccinated.

By extensive serologic investigations it has been shown that the seroimmunity against poliomyelitis is developed rather slowly in the younger age groups in Sweden. Seronegative individuals are found even in the higher ages. Therefore vaccination is needed up to the age of 40.

In Sweden much work has also been devoted to the study of enteric Coxsackie and ECHO viruses. Especially their connection with outbreaks of aseptic meningitis has been investigated.

## SWITZERLAND

PROF. DR O. GSELL

Für die Schweiz ist die epidemische Poliomyelitis heute die wegen ihrer Dauerschäden gefährlichste jährlich wieder auftretende Infektionskrankheit. Die von der Schweizerischen Vereinigung gegen die Poliomyelitis und vom Eidgenössischen Gesundheitsamt gemachten Erhebungen ergeben folgende Resultate:

# MORBIDITÄT

Die Poliomyelitis in der Schweiz ist seit 1930 in stetem Zunehmen begriffen. Die Zahl der gemeldeten Fälle betrug in den Jahren 1930 1930 im Durchschnitt 37 Fälle pro 100 000 Einwohner in den Jahren 1951 1955 erreichte der Durchschnittswert bereits 196 Fälle pro 100 000 Einwohner (absolute Zahlen 1934 1678 1955 919 1956 913)

im Mittelland und die relativ geringe Morbidität in der Westschweiz im Alpengebiet und in der Sudschweiz. Es wurde festgestellt dass im allgemeinen in den Städten weniger Krankheitsfälle auftreten als in den umliegenden ländlichen Bezirken. Die grössten Morbiditätsunterschiede bestehen zwischen dünn und dicht besiedelten Landkantonen. In den letzteren überschritt der Durchschnittswert der letzten 5 Jahre 25 Fälle 100 000 Einwohner

# MORTALITÄT

Die Sterbefälle an Poliomyelitis haben in den letzten 30 Jahren ebenfalls deutlich zugenommen jedoch nicht im gleichen Umfang wie die gemeldeten Fälle. Dieser Umstand ist auf die Tatsache zurückzuführen dass im letzten Jahr zehn vermehrt aparytischen Fällen gemeldet wurden. Die durchschnittliche Mortalität an Poliomyelitis in den Jahren 1930-1934 betrug 0.16 in den Jahren 1951-1955 jedoch bereits 1.66 pro 100 000 Einwohner (absolute Zahlen 1934 114 1955 60 1956 59)

# LETALITÄT

Die Letalität d.h. das Sterberisiko den an Poliomyelitis erkrankten Personen hat sich von 1918 in den Jahren vor 1930 auf 2% in den letzten 3 Jahren vermindert. Dieser scheinbare Rückgang der Letalität ist fast ausschliesslich durch das bessere Meldewesen und durch das Fehlen von aparytischen Fällen bedingt.

# ALTERSVERTEILUNG

Die Altersverteilung der Krankheits und Sterbefälle für die Jahre 1951 1955 war die folgende:

von den an Poliomyelitis erkrankten Personen waren	
55% Kinder unter 10 Jahren	
33% Jugendliche von 10-19 Jahre	
12% Erwachsene über 20 Jahre	
von den an Poliomyelitis gestorbenen Personen waren	
40% weniger als 10 Jahre alt	
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# GEOGRAPHISCHE VERTEILUNG

Die Verteilung der Poliomyelitisfälle innerhalb der Schweiz war sehr ungleich. Auffällig ist eine hohe Morbidität in der Ostschweiz und

# VIRUSISOLIERUNGEN

Bern (Dr. Kersch) in Genf (Dr. Bonitas) in Zürich (Dr. Lindemann) zeigen eine deutliche Prädominanz des Typus I des Poliomyelitisvirus. Bei den an Poliomyelitis erkrankten und Kontaktpersonen findet man am meisten Typ-I Virus in Stuhl nur in einem kleinen Prozentsatz Typ 2 und Typ 3. Unter Stuhl durch Kersch (1955) und 1956 fand sich Typ 1 in 90.3% Typ 2 in 5.0% Typ 3 in 4.7%

# INTEKORPER CECHE POLIOMYELITIS

Zur Beurteilung der Seuchentlage sind serologische Untersuchungen auf Viruskörper unerlässlich. Es wurden in der Schweiz in verschiedenen Orten derartige Untersuchungen in grosser Ausmasse durchgeführt. Die Ergebnisse tabellarisch dargestellt und zeigen dass in Gemeinden die seit 1930 frei von Poliomyelitisviren geblieben sind ein grosser Prozentsatz der Kinder keine Antikörper gegen Typ I Poliomyelitisvirus aufweisen. In Gemeinden die viel Fälle seit 1930 zu verzeichnen haben erreicht die Durchsuchung der Jugendlichen von 5-15 Jahren bereits Werte von über 60% jedoch nur gegen Typ I. Ganz allgemein haben bis zum 1. Altersjahr nur wenige Prozent der Kinder Antikörper gegen alle 3 Typen. Die serologischen Untersuchungen unterstützen die Annahme dass Typ I als hauptsächlichste Ursache paralytischer Erkrankungen in Frage kommt während die häufig serologisch nachgewiesen aber bei Typ I kranken weit seltener als Typ I gefunden werden.

# SCHUTZIMPFUNG

Da durchschnittlich weniger als 50% der Kinder unter 10 Jahren und weniger als 75%

La proportion de décès pour 100 malades montre la gravité de l'épidémie de 1956. La fréquence élevée de ces décès en milieu urbain comparativement au milieu rural tient en grande partie au nombre plus élevé des malades de plus de 15 ans en milieu urbain.

Avant de terminer ce court exposé, je voudrais mentionner quelques résultats de nos examens, effectués à l'Institut Dr J. Cantacuzène sur plus de 550 fèces provenant de malades ou de leur entourage pour la mise en évidence du virus et la détermination des types.

Nous avons isolé 114 souches dont

110 Type 1	(96.49%)
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Nous n'avons jamais rencontré la coexistence avec les virus Coxsackie.

Je me permets en terminant ce rapport de féliciter et remercier au nom de notre Gouvernement l'Association Nationale pour la Poliomyélite des États-Unis d'Amérique pour l'organisation de cette quatrième Conférence et d'exprimer la gratitude de notre pays pour l'hospitalité qui nous a été offerte par la Suisse.

## SWEDEN

DR GUNNAR OLIN

A severe outbreak of polio occurred in Sweden in 1953 with 47 paralytic cases per 100 000 during the epidemic year from April 1953 through March 1954. Since then the polio incidence has been low and slowly decreasing. In 1954 there were only 5 cases per 100 000, in 1955 4 cases, in 1956 3.5 cases per 100 000.

The big outbreak of 1953 offered extensive opportunities for clinical studies of the disease and for investigations concerning its treatment. The Stockholm hospital for epidemic diseases has made an important contribution in this field.

Much interest has also been devoted to the problem of vaccination against polio by a team in Stockholm. A Swedish polio vaccine produced from virus cultivated on human embryonic tissue was tried on 2 000 children early in 1955. No untoward reactions were observed. However, the Cutter incident as well as findings in Swedish control tests necessitated improvement of the safety of the vaccine. It has

also been considered important to try to increase the immunizing capacity of the vaccine. Owing to the work connected with the solution of these problems, large scale production has been retarded.

Therefore the first immunization program had to be carried out mainly with vaccine from the USA, released by courtesy of the federal authorities. When the immunizations started in February of this year only about 100 liters of Swedish vaccine produced from virus cultivated on monkey tissue were available. The program included children aged 4 to 11 years. About 900 000 children were offered vaccination free of charge, about 700 000 accepted and by now have received 2 inoculations each of 1 ml, 3 weeks apart. The program has been accomplished without an accident.

The immunizing capacity of the vaccines used has been titrated both in children and in guinea pigs. The result is presented at the Scientific Exhibit of the Conference.

Since more Swedish vaccine became available in May it has been possible to extend the vaccination to certain categories at risk, namely the staffs of virus laboratories and hospitals for epidemic diseases, pregnant women and on a smaller scale people aged below 35, at their own expense. The conscripts on duty in the armed forces have also been vaccinated.

By extensive serologic investigations it has been shown that the seroimmunity against poliomyelitis is developed rather slowly in the younger age groups in Sweden; seronegative individuals are found even in the higher ages. Therefore vaccination is needed up to the age of 40.

In Sweden much work has also been devoted to the study of enteric, Coxsackie and ECHO viruses. Especially their connection with outbreaks of aseptic meningitis has been investigated.

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Für die Schweiz ist die epidemische Poliomyelitis heute die wegen ihrer Dauerschäden gefährlichste jährlich wieder auftretende Infektionskrankheit. Die von der Schweizerischen Vereinigung gegen die Poliomyelitis und vom Eidgenössischen Gesundheitsamt gemachten Erhebungen ergeben folgende Resultate:

### MORBIDITÄT

Die Poliomyelitis in der Schweiz ist seit 1930 in stetem Zunehmen begriffen. Die Zahl der gemeldeten Fälle betrug in den Jahren 1976-1930 im Durchschnitt 37 Fälle pro 100 000 Einwohner in den Jahren 1951-1955 erreichte der Durchschnittswert bereits 196 Fälle pro 100 000 Einwohner (absolute Zahlen 1954 1 678 1955 919 1956 923).

### MORTALITÄT

Die Sterbefälle an Poliomyelitis haben in den letzten 30 Jahren ebenfalls deutlich zugenommen, jedoch nicht im gleichen Umfang wie die gemeldeten Fälle. Dieser Umstand ist auf die Tatsache zurückzuführen, dass im letzten Jahr zehn vermehrt apasalytischen Fällen gemeldet wurden. Die durchschnittliche Mortalität an Poliomyelitis in den Jahren 1976-1930 betrug 0.16 in den Jahren 1951-1955 jedoch bereits 1.66 pro 100 000 Einwohner (absolute Zahlen 1954 114 1955 60 1956 59).

### LETALITÄT

Die Letalität d.h. das Sterberisiko den an Poliomyelitis erkrankten Personen hat sich von 21.8 in den Jahren vor 1930 auf 7 % in den letzten 3 Jahren vermindert. Dieser scheinbare Rückgang der Letalität ist fast ausschliesslich durch das bessere Meldewesen und durch das Melden von apasalytischen Fällen bedingt.

### ALTERSVERTEILUNG

Die Altersverteilung der Krankheits- und Sterbefälle für die Jahre 1951-1955 war die folgende:

- von den an Poliomyelitis erkrankten Personen waren
- 55.6 % Kinder unter 10 Jahren
- 23.7 % Jugendliche von 10-19 Jahre
- 20.7 % Erwachsene über 20 Jahre
- von den an Poliomyelitis gestorbenen Personen waren
- 40.0 % weniger als 10 Jahre alt
- 30.4 % zwischen 10-19 Jahre und
- 29.6 % 20 Jahre alt und mehr

### GEOGRAPHISCHE VERTEILUNG

Die Verteilung der Poliomyelitisfälle innerhalb der Schweiz war sehr ungleich. Auffällig ist eine hohe Morbidität in der Ostschweiz und

im Mittelland und die relativ geringe Morbidität in der Westschweiz, im Alpengebiet und in der Sudschweiz. Es wurde festgestellt, dass im allgemeinen in den Städten weniger Krankheitsfälle auftreten als in den umliegenden ländlichen Bezirken. Die grossen Morbiditätsunterschiede bestehen zwischen dünn und dicht besiedelten Landkantonen. In den letzteren überschritt der Durchschnittswert der letzten 5 Jahre 25 Fälle/100 000 Einwohner.

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Virusisolierungen in Cenf (Dr. Bonifas) in Bern (Dr. Kreh) in Zürich (Dr. Lindemann) zeigen eine deutliche Prädominanz des Typus 1 des Poliomyelitisvirus. Bei den an Poliomyelitis erkrankten und Kontaktpersonen findet man am meisten Typ-1 Virus in Stuhl, nur in einem kleinen Prozentsatz Typ 2 und Typ 3. Unter 279 isolierten Poliomyelitisstämmen aus dem Stuhl durch Kreh (1955 und 1956) fand sich Typ 1 in 90.3 % / Typ 2 in 5.0 % Typ 3 in 4.7 %.

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Zur Beurteilung der Seuchenlage sind serologische Untersuchungen auf Abwehrkörper unerlässlich. Es wurden in der Schweiz an verschiedenen Orten derartige Untersuchungen in grossem Ausmass durchgeführt. Die Ergebnisse, welche in der wissenschaftlichen Ausstellung tabellarisch dargestellt sind, zeigen, dass in Gemeinden, die seit 1950 frei von Poliomyelitis fallen geblieben sind, ein grosser Prozentsatz der Kinder keine Antikörper gegen Typ 1 Poliovirus aufweisen. In Gemeinden, die viel Fälle seit 1950 zu verzeichnen haben, erreicht die Durchseuchung der Jugendlichen von 5-7 Jahren bereits Werte von über 60 %, jedoch nur gegen Typ 1. Ganz allgemein haben bis zum 7. Lebensjahr nur wenige Prozent der Kinder Antikörper gegen alle 3 Typen. Die serologischen Untersuchungen unterstützen die Annahme, dass Typ 1 als hauptsächlicher Erreger paralytischer Erkrankungen in Frage kommt, während die Typen 2 und 3 in der Bevölkerung fast ebenso häufig serologisch nachgewiesen, aber bei Erkrankten weit seltener als Typ 1 gefunden werden.

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23.7 Jugendliche von 10 bis 19 Jahre

20.7 Erwachsene über 20 Jahre

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Die Verteilung der Poliomyelitisfälle inner halb der Schweiz war sehr ungleich. Auffällig ist eine hohe Morbidität in der Ostschweiz und

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der Jugendlichen von 10 19 Jahren Antikörper gegen Poliomyelitisvirus Typ 1 besitzen ist es in der Schweiz empfehlenswert bei allen Jugendlichen Schutzimpfungen vorzunehmen. Die Tatsache dass Dreiviertel der Poliomyelitispatienten der letzten 5 Jahre Jugendliche unter 20 Jahren waren spricht zwingend für eine Impfpriorität dieser Bevölkerungsgruppe.

Die Impfungen mit der Salk Vakzine sind ab Ende 1956 in grossem Umfange durchgeführt worden und zwar nach Impfprogrammen die in den einzelnen Kantonen beträchtlich variierten ganz allgemein aber die Altersklassen zwischen 2 und 14 Jahren berücksichtigten. Die Impfungen erfolgten zum Teil gratis zum Teil mit partieller finanzieller Beteiligung des Impflings. Öffentliche Impfaktionen waren stets freiwillig und ergaben eine Teilnahme zwischen 50 und 90 % in den für die Impfung vorgesehenen Altersklassen.

An Poliomyelitisvakzine wurde bis 15 Mai 1957 eingeführt 1764 Lit r und in der Schweiz selbst hergestellt 525 Liter. Dieser Impfstoff reichte aus um mehr als 850 000 Personen zweimal zu impfen. Man kann annehmen dass bis Sommer 1957 58 % aller Jugendlichen unter 18 Jahren in der Schweiz zweimal geimpft wurden. Ueber die Ergebnisse kann erst 1958 berichtet werden.

#### HILFE FÜR DIE POLIOMYELITISGESCHÄDIGTEN

Für die schweren Atmungsstörungen bei Poliomyelitis stehen in der Schweiz heute 130 moderne Respiratoren zur Verfügung darunter 30 Engstrom Apparate. In den verschiedenen Klinikzentren sind Equipen zur Behandlung von Atmungsgelähmten geformt worden. Am 1 April 1957 fanden sich 54 Kranke die total oder zeitweise auf Apparate angewiesen waren. Für die Behandlung im akuten Stadium wie auch für die sachgemässe systematische Nachbehandlung fanden Kurse durch die Schweizerische Vereinigung gegen die Poliomyelitis statt. Es stehen eine Reihe besonderer orthopädischer Institute und Badekurorte zur Verfügung.

Gegen die materiellen Folgen der Kinderlähmung sind heute 28 Millionen Personen (bei rund 5 Millionen Einwohnern der Schweiz) im Schweiz Verband für die erweiterte Krankenversicherung sowie eine weitere beträchtliche Zahl bei zwei privaten Ver-

sicherungsgesellschaften speziell gegen Kinderlähmungsfolgen versichert, mit einer Entschädigung zwischen 20 50 000 Franken abgestuft nach Altersgruppen. In einzelnen Kantonen besteht eine besondere Invalidenversicherung, in Bearbeitung ist eine schweizerische Invaliditätsversicherung. Für die Wiedereingliederung der Gelähmten kümmern sich speziell zwei grosse Organisationen: Pro Infirmis und Schweiz Arbeitsgemeinschaft zur Eingliederung von Behinderten in die Volkswirtschaft.

#### STATISTISCHE ANGABEN

Detaillierte Zahlenangaben über das Vorkommen der Poliomyelitis in der Schweiz und über die Verteilung des Antikörpergehaltes in der Bevölkerung auf die verschiedenen Kantone über Institutionen und Versicherungen finden sich in der wissenschaftlichen Ausstellung auf 27 Tafeln zusammengestellt mit Hilfe der Schweiz Vereinigung gegen die Poliomyelitis und des Eidgenössischen Gesundheitsamtes.

#### THAILAND DR C PURANANANDA

The disease was first recognized about 30 years ago when the Medical School was reorganized with the aid of the Rockefeller Foundation. Since then a few cases among children were reported each year. The diagnosis was made on the history of the illness and the symptom of paresis which was temporary complete recovery following after a few months. There was no mortality therefore it was considered to be a mild infection like coryza. After World War II the number of immigrants from Europe and America increased and formed an important colony in the capital of Thailand. In 1950 and 1951 cases of poliomyelitis were reported in Singapore which is one of the nearest ports connected to Bangkok by sea rail and air. Attempts were made to safeguard the children and the foreign communities and no cases were reported in Bangkok.

In September 1952 the first case of poliomyelitis was reported in a Swiss lady who died after 4 days of illness. A few days later a Danish lady who was expecting a child contracted the disease. She was rushed to Singapore and was placed in an iron lung until the birth of her son. Between September and December 1952 there

were 388 cases reported in Bangkok. This included all age groups foreigners as well as Thai with a mortality of 6 per cent. Diagnosis was made by clinical examination and the treatment was symptomatic. An iron lung was acquired in the latter part of the epidemic. When the epidemic was over there were quite a few cases of paralysis but no cases of spinal column involvement.

It is considered that this was the first epidemic of poliomyelitis in Thailand. A few epidemiologists tried to find reasons for it and came up with the following hypotheses:

1. The improvement of sanitary conditions in our country.
2. The introduction of new strains.
3. The transmission of old strains into a new fertile soil.

The improvement of sanitary conditions was ruled out by the fact that there were no remarkable improvements since the war. Some public utilities were not as good as in the pre-war years; therefore the inhabitants still stand a good chance of contracting small repeated infections.

As for the second theory by sampling blood of aged inhabitants of a province situated 100 km. east of Bangkok where no cases of polio were reported during the epidemic, it was found that the 3 types of polio virus already existed in Thailand.<sup>3</sup> Samples of blood from children between the ages of 2 to 7 who were convalescing from this epidemic of polio were also tested. The result was that nearly all of the samples showed antibody against Types 1, 2 and 3 but antibody to Type 3 was less frequent.<sup>2</sup> The results of these 2 analyses strengthen the theory that there is constant mild infection taking place in the people.

The third hypothesis that of local strains being introduced into a fertile soil needs more experimentation to be proved.

Attempts were made to establish a virus laboratory with facilities for making possible early diagnoses. However these attempts have been in vain because of the shortage of all factors from virologists, technicians and trained employees to equipment. The latter is easier to acquire than the first three. A few foreign organizations and institutions have promised aid but nothing has materialized as yet.

The report of the success of Salk vaccine from

the United States has aroused Thai medical scientific and educational workers to consider whether the use of Salk's vaccine is indicated in Thailand.<sup>4</sup> A review of the report of the Conference of WHO Experts held in Stockholm in November 1955 together with the results of experiments on polio antibody in Thai adults and children show that Salk vaccine is not indicated. Also since the epidemiology and the mode of infection of polio resembled typhoid fever there are a few cases of poliomyelitis yearly and if the epidemic cycle will be the same as in other countries we are expecting another outbreak in September of this year. However as yet nothing has been done except the improvement of the water supply in Bangkok. Information should be distributed to the public accordingly.

It is of interest to compare countries where poliomyelitis is most dangerous with countries where the disease is less dangerous and with those countries where polio is not dangerous. In the first group are the United States of America, Scandinavia and Switzerland. The second group is composed of most of the countries in Europe and Canada. The third group comprises the Near East and the Far East countries. The countries in the first group are those with the best sanitary conditions, their people work very hard and the amount of sunshine through the year is not enough to attenuate the virus. The countries in the second group have a comparatively lower standard of sanitary conditions, their people work very hard and the amount of sunshine is comparatively less through the year. In a few countries in this category there are other virus infections such as influenza. The countries of the third group have poor sanitary conditions and a multitude of bacteria flourish thus hampering the thriving of polio virus.<sup>4</sup> Their people are not hard working. There is a lot of sunshine throughout the year, the amount of ultraviolet in the sunshine attenuates the viruses<sup>1</sup> and they become less virulent.

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## UNION OF SOVIET SOCIALIST REPUBLICS

DR VALENTIN D SOLOVIEV

In the Soviet Union the marked increase in the incidence of poliomyelitis was first noted in 1955 when the morbidity rate reached a figure never previously observed namely 8 cases per 100 000 inhabitants. Approximately the same figure was observed in 1956. In 1957 the incidence seems not to decline as we may judge by the available information.

A summer-autumn seasonal prevalence of poliomyelitis was pronounced in 1955 and 1956 the peak of the incidence being in July-August-September and October.

Distribution by age groups shows that young children under 7 are affected most often they make up about 85 per cent of cases.

However in some western regions of the USSR we see a different age group distribution. In the Estonian Soviet Socialist Republic for instance about 15 to 20 per cent of cases were adults of 20 or more years of age.

The average lethality of poliomyelitis in 1955 was 37 per cent and in 1956 2.6 per cent.

Typing of the strains isolated in 1956 showed the following distribution of virus types: 63 per cent of the strains belonged to Type 1, 12 per cent to Type 2 and 25 per cent to Type 3. Moreover from stool specimens of patients and their contacts a number of strains were isolated which were untypable when tested with specific sera of all 3 types of polio virus.

For the control of poliomyelitis two institutes have been established: the Poliomyelitis Institute of the Academy of Medical Sciences of the USSR aiming at research work in the problem and the Moscow Institute of the Ministry of Health of the USSR mainly intended for the manufacture of Salk vaccine.

Laboratories have been set up in large towns

of the USSR for the diagnosis of poliomyelitis.

Great attention is given to treatment of poliomyelitis patients. Besides special departments in children's hospitals there are centers for rehabilitation therapy and special sanatoria.

The treatment of poliomyelitis patients is free of charge as are all medical services in the USSR.

## UNITED KINGDOM (Colonies)

DR W H BRADLEY

The available evidence indicates that poliomyelitis must be regarded as being endemic in practically all the British Colonial Territories overseas. In the majority of the territories the incidence of paralytic cases is sporadic but outbreaks of epidemic type and proportions occur in circumstances which at present are unpredictable. Reference was made to some of these at the Third International Conference. Several have occurred since that date and are referred to here under the name of the individual territory affected.

The precise immunologic state of the population of the overseas territories is not known. Evidence derived from studies of the incidence of the disease indicates that a fair degree of immunity is acquired by the local communities by the age of 5 years but that among immigrants from Europe and the western countries older children and young adults are susceptible and among them the incidence rate is disproportionately high. In the Kenya epidemic of 1954 the racial incidence rates per 100 000 were: Europeans 254, Asians 465, Africans 65. These figures are fairly typical of racial incidence in most of the African Colonial Territories.

Pilot serologic surveys have been carried out in several territories. The results reported from Lagos, Nigeria, showed that of 26 sera examined only 1, a child of 2 years, was negative for all 3 types. In Singapore 40 per cent of children of local origin in the 5 to 9 age group had antibodies to all 3 types. Similar findings have been reported from Uganda. In the Far East an extensive survey sponsored by the World Health Organization is being carried out by Professor Hale of the University of Malaya. This will include Hong Kong. The Federation of Malaya

Singapore Borneo and Sarawak and the work when completed will give a much more accurate picture than can be deduced at present.

**Jamaica** The epidemic which started in June 1954 was fading out early in 1955. In a population of approximately 1,500,000 792 cases were notified up to February 5 1955. In 1956 the incidence had returned to endemic proportions. This was the first serious epidemic reported on the island.

**Trinidad** There is a record in this island of several epidemics in recent years: 1937 106 cases 1941 59 cases 1942 135 cases 1945 99 cases. In 1954 an epidemic coincided with that in Jamaica 196 cases were notified of which 50 per cent were under 5 years of age and 163 under 16 years. There are indications of an other outbreak occurring in 1957 but full details are not yet available.

**Tanganyika** Poliomyelitis has occurred in epidemic form as follows: in 1954 170 cases 1955 123 cases 1956 458 cases. A feature of these outbreaks in this large territory with a very low population density has been the localized nature of the outbreaks. In 1956 there were 3 distinct areas affected without a general spread to the larger urban centers. This outbreak was continuing in 1957.

Vaccination programs are being planned in many territories and in some have already been developed on a considerable scale. The full development of vaccination programs obviously must be influenced by the availability of vaccine and internal economy. The accessibility of low density populations is also a controlling factor. Apart from these practical considerations it is realized that more precise information is required as to the immunologic state of communities and age groups. It is felt that this will require further investigation before anything approaching mass vaccination campaigns can be justified scientifically. Meanwhile programs are being framed to offer protection to those who can reasonably be assumed to be at greatest risk.

## URUGUAY

PROF. ENRIQUE M. CLAVEAUX

La poliomyelitis en el Uruguay diagnosticada desde 1905 se ha mantenido presente desde esa fecha hasta ahora en forma de brotes anuales de intensidad variable. Los empujes más im-

portantes fueron los de los años 1936-1937 con 284 casos 1943 con 167 casos 1945 con 165 casos y 1954-1955 con 574. El último empuje configuró una verdadera epidemia siendo evidente que la enfermedad ha adquirido ya una importancia epidemiológica alarmante.

La enfermedad predomina en los meses calurosos del año. Excepcionalmente aparece en el invierno como ha ocurrido en la ciudad de San José (27 casos entre Abril y Septiembre).

Las estadísticas del país consignan solo las formas paralizantes de la enfermedad. En la última epidemia la morbilidad fue de 20 por 100,000 habitantes.

La mortalidad en esa misma epidemia fue de 67 %.

La mayor morbilidad correspondió a niños hasta los 3 años (44 %).

La morbilidad para mayores de 14 años representó el 14 %.

Las formas respiratorias (11 %) tuvieron una mortalidad de 50 %.

Las formas graves respiratorias y parálisis extendidas representan 50 % del total.

El país ha realizado en los tres últimos años un esfuerzo marcado en la lucha contra la parálisis infantil. Se ha organizado un servicio autónomo dedicado exclusivamente al tratamiento de las formas agudas. Se dispone de 47 pulmones de acero y 4 aparatos de presión positiva (Engstrom) utilizándose además bombas y corazas. El control de la capacidad vital y aire corriente se hace en forma satisfactoria con aparatos adecuados.

Los servicios complementarios de rayos X laboratorio y fisioterapia atendidos por especialistas funcionan eficientemente.

El público ha respondido al llamado de las autoridades y sigue contribuyendo ampliamente para la instalación de servicios de recuperación.

La vacunación por el método Salk actualmente se aplica en el país en forma voluntaria y en las condiciones técnicas universalmente admitidas como de rigor.

Se practica en tres inyecciones—las dos primeras a un mes de distancia entre ellas y la tercera al 6 mes de la segunda.

La vacuna ha sido en general bien tolerada.

El número actual de vacunados es de 63,466 con la primera dosis y de 50,408 con 2 dosis.

Se ha vacunado preferiblemente a niños entre 6 meses y 6 años de edad (46,911) de los cuales

38 000 recibieron tambien la segunda dosis

La vacunacion realizada fuera de periodo epidemico no ha podido traducirse en hechos que permitan juzgar su eficiencia

En lo que va transcurrido del presente ano solo se han producido en el Uruguay 25 casos de paralis. Dos de estos casos estaban vacunados uno con una dosis y otro con dos dosis

Aun no se ha comenzado a aplicar la tercera inyeccion

Consideramos que un juicio sobre la calidad de los resultados obtenidos por la vacunacion solo podria en nuestro pais realizarse cuando hayamos vacunado toda la poblacion infantil con las tres dosis como la vacunacion no es obligatoria dependera en gran parte de la aceptacion del publico poder llegar a aquel desideratum

Creemos que si la inmunidad tiene caracter precario (corta duracion) la necesidad de repetir las inoculaciones alejara al publico de los dispendios Si se agrega a esto el caracter parcial de la vacunacion no obligatoria estimamos que sera muy dificil que por el metodo Silk se logre hacer desaparecer la paralis infantil en nuestro pais

## YUGOSLAVIA

PROF. K. TODOROVITCH

Pendant longtemps jusqu'en 1928 la poliomyelite en Yougoslavie existait a l'etat sporadique Jusque la on n'avait note que des cas isolés tant chez des enfants que chez des adultes Jusque en 1940 le nombre des malades etait petit moins de 100 par an excepte l'annee 1928 ou le nombre des malades atteignit le chiffre de 201 Pendant cette periode la poliomyelite etait accompagnée d'une mortalite tres élevée la quelle certaines années atteignait meme 21 / (1929) 23 % (1940) 30 % (1934) et 33 % (1929) Le nombre des malades par rapport au

nombre des cas mortels etait en proportion inverse

A partir de 1946 le nombre des malades atteints de la poliomyelite va en augmentant sans quelque regularite Au cours des 10 dernieres années le nombre etait en moyenne 2 fois plus grand qu'au cours des 2 periodes de 10 ans precedentes demontrant meme en certaines années des augmentations notables comme cela fut le cas par exemple en 1953 quand 715 cas furent enregistres dont 41 etaient mortels (mortalite = 5.73 %) Au cours des 2 années suivantes l'on enregistra un nombre 2 fois moins grand de malades de la poliomyelite soit 339 en 1954 avec mortalite de 7.96 / et 375 en 1955 avec mortalite de 4.31 / L'annee passée en 1956 le nombre des cas enregistres en Yougoslavie a atteint le chiffre de 1254 dont 53 etaient ete mortels (létalite = 4.22 %)

Au cours des 30 dernières années la poliomyelite en Yougoslavie se manifestait presque toujours a l'etat sporadique et ce n'est que certaines années qu'elle prenait un caractere d'épidemie mais toujours faible d'envergure

Le tableau 19 indique la repartition des malades par republique telle qu'elle se presentait en 1956 année au cours de laquelle fut noté le plus grand nombre de cas

La poliomyelite ne se manifestait que dans certaines regions delimitées sans qu'il y ait eu tendance a sa propagation par contacts et sans qu'en ces endroits le nombre de malades avec des paresthesies ou des paresthesies manifestes ait ete note au sein des memes collectivites par exemple au sein de la meme famille parmi les élèves d'une meme classe parmi les habitants d'une meme rue etc

Au point de vue mortalité la Yougoslavie se trouve parmi les pays ou la mortalité par suite de la poliomyelite est des plus faibles

TABLEAU 19 REPARTITION DES MALADES PAR RÉPUBLIQUE 1956

RÉPUBLIQUE POPULAIRE	NOMBRE D'HABITANTS	MALADES	CAS MORTELS
Serbie	7 335 000	754	22
Croatie	4 043 000	112	4
Slovénie	1 516 000	260	24
Bosnie et Herzégovine	3 084 000	67	1
Macédoine	1 417 000	56	2
Monténégro	458 000	5	—

Comme dans les autres pays européens la poliomyélite en Yougoslavie a le caractère de maladie saisonnière. Au cours des derniers mois de l'automne au cours de l'hiver et du printemps le nombre des malades était très petit et n'augmentait sensiblement que pendant l'été. Cette régularité saisonnière a été notée indépendamment du nombre des malades.

La plupart des malades en Yougoslavie sont des enfants âgés de 1 à 3 ans. Pour les nourrissons le nombre des cas était toujours légèrement inférieur. Dans les régions où la poliomyélite apparaît comme maladie épidémique la mortalité est légèrement supérieure chez les adolescents de 14 à 15 ans à celle chez les enfants plus jeunes ou chez les adultes. En 1956 année où fut noté le nombre de cas le plus grand le malade le plus jeune était un nouveau-né de 2 semaines et le plus âgé un homme de 56 ans.

La plupart de nos malades provenaient des campagnes (1610). On n'a pas pu établir de lien épidémiologique convaincant entre différentes agglomérations éloignées ou proches ni la source commune possible de l'infection ni la même voie ou manière de la diffusion ni la

L'analyse des cas qui se sont manifestés uniquement sur un territoire sur lequel vivent 500 000 d'habitants classes d'après les dates de la maladie démontre une dispersion telle qu'elle ne permet d'établir entre eux aucun lien quelque douteux qu'il soit. Même dans les grandes villes et agglomérations de 50 à 100 000 habitants ou dépassant 500 000 ce n'est relativement que très rarement qu'on a pu noter l'apparition de la maladie le même jour chez un nombre plus grand de personnes sur un territoire donné. L'année passée la maladie avait au cours de la période de l'été la caractéristique d'épidémie dans certaines agglomérations industrielles. Toutefois même à cette occasion l'apparition de la maladie chez 2 ou plusieurs personnes appartenant au même établissement n'était qu'exceptionnelle.

Dans les localités où la poliomyélite avait pris des proportions épidémiques l'on n'a pu chez les animaux domestiques relever aucune autre ou semblable maladie à caractère sporadique ou épidémique.

Il y a lieu de mentionner que l'apparition en plus grand nombre de la poliomyélite dans certaines régions de Yougoslavie avait été précédée généralement chez les enfants et adolescents d'affections fébriles de courte durée avec des signes manifestes de méningite et de méningo-encéphalite et avec de l'albuminorachie et pleocytose modérées dans le liquide céphalo-rachidien. L'affection se passait sans leucocytose dans le sang et sans qu'en ait pu être relevée la cause parmi les microbes pathogènes connus. Les recherches virologiques sont restées sans résultats sur

Lors de l'épidémie qui a eu lieu dans la République de Serbie on a noté un grand pourcentage de malades chez lesquels se trouvait la paralysie du V facialis soit comme l'unique soit comme l'un des signes accompagnant les lésions nerveuses périphériques. La proportion était de 23 (41.63%) des cas indubitables de poliomyélite.

Des recherches virologiques d'entrevue limitée ont démontré qu'en Serbie par exemple sur 78 virus identifiés les 3/4 appartenaient au type 1 environ 7 / au type 2 et 1/4 au type 3.

Les recherches sérologiques menées sur un territoire limité ont démontré que les anticorps pour les type 2 se trouvent chez les enfants de moins de 2 ans dans une proportion de 60% chez les adolescents et les adultes presque à 100%.

D'après ces résultats on dirait que les enfants des campagnes démontrent plus lentement et plus tard la présence d'anticorps dans le sang que les enfants des villes.

Les anticorps pour le type 3 du virus polio apparaissent plus tard et plus lentement. Les anticorps pour le type 1 sont les moins représentés.

Note The Reports published are those for which requested texts were received



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# Vaccination Against Poliomyelitis

MONDAY AFTERNOON, JULY 8, 1957

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DR J ØRSKOV  
Statens Seruminstitut  
Copenhagen

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Pittsburgh

DR ALEXANDER D LANGMUIR  
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DR DAVID BODIAN for the Technical  
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## *Discussants*

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Stockholm

DR JAMES H S GEAR  
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# Basic Principles Underlying Immunization Against Poliomyelitis with a Noninfectious Vaccine

DR JONAS E SALK

At the International Poliomyelitis Congress in Rome in 1954 a summary was presented of information then available relevant to vaccination with a killed virus vaccine. For this Congress a similar effort is being made but as is to be expected the questions before us are different from those of 3 years ago. Principal interest now centers around vaccine effectiveness on the practical questions of the degree and the duration of immunity and the more fundamental questions concerning the mechanisms of immunity.

In considering which parts of the whole to discuss and how best to present the pertinent facts and the inferences that have been drawn therefrom it seemed to me that we might begin by making an admittedly erroneous supposition namely that all humans possess exactly the same number of antibody forming cells and that these cells are all equally responsive immunologically.

The first example is that of a child in whom the antibody persistence pattern after primary immunization is as illustrated in Figure 32. If all children similarly treated responded in this

way, it would be generally agreed that they would be immune to paralysis at least for the period of time here indicated and that in 3 years. Figure 33 depicts a child in whom reinoculation was performed 7 months after primary vaccination and the same inference in regard to persistence of immunity to paralysis could be drawn in this instance and that is 4 years.

Additional examples of persistence of antibody over a 3½ year period are illustrated in Figure 34. The 2 children used for illustration were vaccinated first in May 1953 and a secondary or booster dose was given in December 1953. The results of serologic tests on 9 blood samples are shown. The first 7 black symbols in the case of child F 297 on the left represent the results of simultaneous serologic testing. The seventh blood sample was retested along with No. 8 when the latter was obtained at 2½ years and No. 8 was retested along with No. 9 when the latter was obtained at 3½ years. The actual values for antibody titer obtained each time are plotted and the tendency for persistence of antibody for each type is clearly evident.

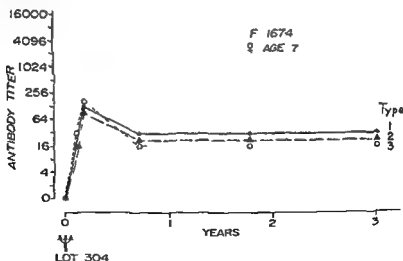


FIG 32 Persistence of antibody 3 years after primary vaccination

TABLE 20 PERSISTENCE OF TYPE 1 ANTIBODY  
OVER 3½ YEAR PERIOD IN CHILDREN  
GIVEN BOOSTER DOSE AT 7 OR 11  
MONTHS AFTER PRIMARY

SUBJECT AGE IN 1953	1½ Yrs	2½ Yrs	3½ Yrs
F 137 13 yrs	512	512 16	16
F 378 10 yrs	256	128 64	64
F 336 12 yrs	128	32 128	32
F-45 9 yrs	128	32 64	32
F 135 11 yrs	128	128 16	32
F 187 9 yrs	32	16 16	16
F 207 9½ yrs	32	16 16	16
F 291 11½ yrs	32	32 16	32
F 207 8 yrs	32	32 32	16
F-47 10 yrs	32	32 4	4
F 27 5 yrs	32	16 16	32
F 136 7 yrs	16	8 0	8
F 69 16 yrs	8	16 4±	4±

TABLE 21 PERSISTENCE OF TYPE 2 ANTIBODY  
OVER 3½ YEAR PERIOD IN CHILDREN  
GIVEN BOOSTER DOSE AT 7 OR 11  
MONTHS AFTER PRIMARY

SUBJECT AGE IN 1953	1½ Yrs	2½ Yrs	3½ Yrs
F 136 7 yrs	256	32 128	128
F-47 10 yrs	64	32 32	32
F 27 5 yrs	64	32 64	32
F 69 16 yrs	32	128 256	64
F 328 10 yrs	32	64 64	64
F-45 9 yrs	3	64 3	32
F 297 9½ yrs	16	16 8	8
F 135 11 yrs	16	8 16	16
F 137 13 yrs	16	32 8	8
F 182 9 yrs	16	8 8	8
F 291 11½ yrs	16	16 16	16
F 207 8 yrs	16	8 8	8
F 336 12 yrs	8	16 16	8

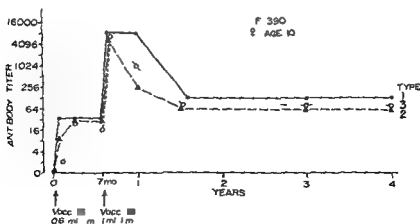


FIG 33 Degree of persistence of antibody over a 4 year period.

TABLE 22 PERSISTENCE OF TYPE 3 ANTIBODY  
OVER 3½ YEAR PERIOD IN CHILDREN  
GIVEN BOOSTER DOSE AT 7 OR 11  
MONTHS AFTER PRIMARY

SUBJECT AGE IN 1953	1½ YRS	2½ YRS	3½ YRS
F 297	512	64	
9½ yrs		256	128
F 136	128	32	
7 yrs		32	32
F 135	32	16	
11 yrs		32	8
F 336	32	32	
12 yrs		16	16
F-47	32	32	
10 yrs		64	64
F 328	32	32	
10 yrs		64	64
F 27	16	32	
5 yrs		64	64
F-45	16	16	
9 yrs		16	16
F 137	16	8	
13 yrs		8	8
F 291	16	16	
11½ yrs		4	4
F 202	16	8	
8 yrs		8	8
F 69	8	16	
16 yrs		16	8
F 182	8	8	
9 yrs		±	4

Additional examples are shown in Tables 20 and 21. In Table 20 we show data for Type 1. The first line in each instance represents results of simultaneous tests for antibody in blood samples drawn 1½ and 2½ years. The second line represents the results of the simultaneous tests for antibody in the 2½ and 3½ year bleedings. The degree of comparison of results of the 2½ year blood tests on the two different occasions shows the degree of comparability that is obtained in repeat tests.

Table 21 shows data of the Type 2 antibody levels and you will observe the tendency for there to be persistence between the 1½ and 2½ year points and the 2½ and 3½ year points in all instances. I have been asked whether or not this might represent maintenance of immunity due to natural infection and I can tell you that in those instances where natural infection has occurred there is usually a rise of antibody to but one type. Table 22 shows the degree of persistence of antibody to the Type 3 virus.

Now from these evidences it seems likely that antibody will be demonstrable in most individuals for a number of years after an effective course of immunization. Therefore the attainment of effective immunization initially is the point of first importance and the achievement of this seems to be dependent on the administration of a sufficient quantity of antigen. It is

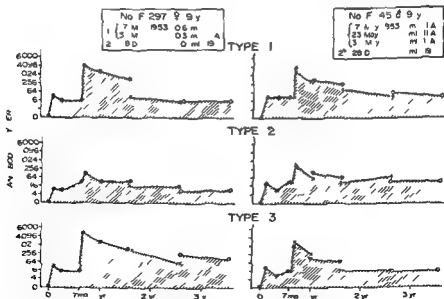


FIG. 34 Degree of persistence of antibody over 3½ years

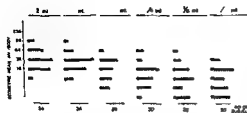


FIG 35 Dosage effect. Type 1 antibody response 2 weeks after 1 dose of Preference Vaccine A given intramuscularly to subjects with no demonstrable preantibody to any type (Salk J E JAMA 158 1239)

self-evident that a minimal number of antigenic units will engage merely a minimal number of antibody forming cells and the amount of antibody induced thereby will also be minimal. A more potent antigen will induce the formation of higher levels of antibody not only because more cells are involved but also possibly because each cell is exposed to more antigen. With more potent antigens saturation of the antibody inducing mechanism is more likely to be approximated as is probably the case in the infectious process where virus multiplication occurs

and where a large number of antibody forming cells are engaged

Data in Figure 35 show that individuals given the same dose of antigen are more responsive than others. From these data it also appears that the response of the individual can be enhanced if the amount of antigen administered is increased at least within limits. And you can see clearly from Figure 35 when a large dose is given all respond to a single dose of vaccine and when a smaller dose of vaccine is given some fail to respond. This dosage response effect can be demonstrated with Types 2 and 3 as well. Thus it is clear that failure of response could result either because of an inadequate or impotent stimulus or because of a relatively poorly responding immunologic mechanism. Therefore the problem seems to be one of adjusting vaccine potency and number and spacing of inoculations to effect sufficient stimulation especially in those individuals who are the least responsive immunologically.

Although the level of antibody observed after vaccination is the resultant of (1) the degree of responsiveness of the individual (2) the amount of antigen administered (3) the spacing be

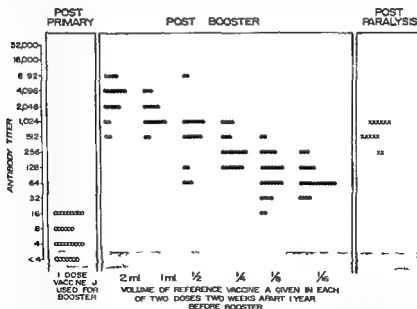


FIG 36 Type 1 antibody response after booster in relation to potency of vaccine used for primary inoculation and as compared with antibody titer following natural infection (Salk J E Am J Pub Health 47 1)

tween inoculations and (4) the time that has elapsed since the last inoculation it is abundantly clear that a demonstrable level of antibody once induced by a killed polio virus vaccine is not necessarily evanescent. With the degree of biologic variation observed in antibody response induced by primary vaccination and in level of antibody present in intervals thereafter or following a booster dose it is clear that the practical problem requires that a sufficient excess of antigen be incorporated in the vaccine to induce the desired effect consistently. If to the problem posed by human variation in responsiveness is added the variation in potency of vaccine preparations it is probable and it is also understandable that the full potential of the procedure has not yet been realized.

Nevertheless decisions must continue to be made and goals must be defined. The objective to be attained can be decided in part through tests made with a reference vaccine of reasonable potency against which unknown vaccines can then be standardized. Thus we have attempted to do with Reference Vaccine A. It has been demonstrated that there is a dosage response relationship not only with respect to the level of antibody induced after primary vaccination as may be seen from Figure 35 but also with respect to the level of antibody induced

after secondary stimulation as is shown in Figure 36.

Let me point out the nature of this experiment. Groups of children were given 2 doses consisting of 2 cc of vaccine 1 cc a half a quarter an eighth or a sixteenth 2 weeks apart, a year before a booster dose was given. The antibody level was then determined 2 weeks after the booster dose. The control group that had not been vaccinated previously and had received the booster dose of vaccine is shown on the left; you will see the way in which those who receive a primary immunization with the different dosages responded to the same booster dose of vaccine that was equal in all groups. You will see clearly that the degree of response to the booster is related to the quantity of antigen administered primarily. Thus we can see that the degree of hyperreactivity induced by the primary immunologic experience is revealed by the degree of response at the time of revaccination.

These findings indicate that the hyperreactivity is not an all-or-none state but that it exists in varying degrees. We shall return to this question in a moment.

Data in Figure 37 indicate the degree to which antibody response was induced and maintained in a group of approximately 150

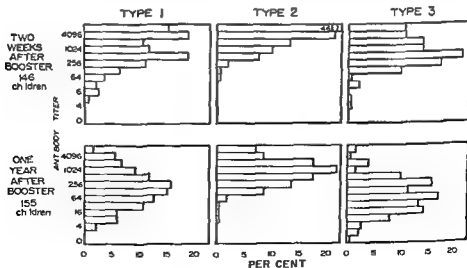


FIG 37 Antibody distribution 2 weeks and 1 year after booster. Primary inoculation with commercial vaccine. Primary 2 doses 1 cc im 2 weeks apart 6 lots 1955 vaccine from 4 producers. Booster 1 cc Vaccine J im 1 year later.

children who possessed no antibody for any type before vaccination. This figure illustrates antibody distribution and the length of the columns indicates the number of individuals in per cent who possess antibody at the different levels shown on the left hand scale 2 weeks after the booster dose in children who had

received 2 doses of 1 cc intramuscularly 2 weeks apart of 6 lots of 1955 commercial vaccine from 4 producers. A year later they received 1 cc of Vaccine J intramuscularly and the levels shown are those observed 2 wks after the booster dose for Type 1 Type 2 and Type 3. You will observe that there are none in the zero range

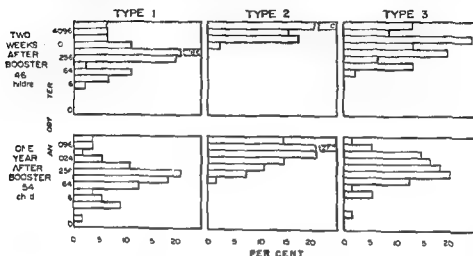


FIG 38 Antibody distribution 2 weeks and 1 year after booster. Primary inoculation with Reference Vaccine A (composite for groups given 2 ml, 1 ml or  $\frac{1}{2}$  ml). Primary 2 doses 1 m 2 weeks apart 2 cc 1 cc or 1 cc 1:2 dil. Reference Vaccine A. Booster 1 cc Vaccine J 1 m 1 year later.

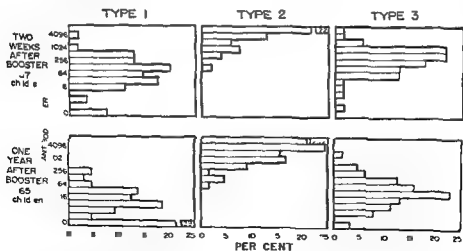


FIG 39 Antibody distribution 2 weeks and 1 year after booster. Primary inoculation with Reference Vaccine A (composite for groups given  $\frac{1}{4}$  ml,  $\frac{1}{8}$  ml or  $\frac{1}{16}$  ml). Primary 2 doses 1 cc 1 m 2 weeks apart 1:4, 1:8 or 1:16 dil. Reference Vaccine A. Booster 1 cc Vaccine J 1 m 1 year later.

and the majority of them are quite high. One year later without any further inoculations antibody determinations were made and you will see the tendency for decline but you will also observe that there are few if any in the zero column. Thus it is clear that there has been a considerable degree of persistence of antibody in those who received commercial vaccine of the 1955 vintage followed by laboratory vaccine for the booster dose.

Figure 38 shows similar data for a group of children (the irregularities in the smoothness of the curve as compared to Fig 37 are due to the smaller size of groups) who had received either 2 cc, 1 cc or one half cc of Reference Vaccine A followed by a booster 1 year later of Vaccine J.

Here again you see 2 weeks post booster the high levels of antibody. You see the tendency for decline but there is still antibody 1 year after vaccination in almost all. The picture is somewhat different in Figure 39 which shows the results observed in a group of children who had had primary immunization with a smaller dose of Vaccine A, a 1:4, 1:8 or 1:16 dilution followed by the booster. You will see that some of them did not respond to Type 1 even to the booster dose when their primary sensitization was with this level of antigen. For Type 2

and Type 3 there was a fair response. But at the end of 1 year there is a tendency for antibody levels to decline so that 29 per cent had no Type 1 antibody that was detectable in a group of 65 children. The Type 2 levels were high and Type 3 was somewhat intermediate.

Figure 40 shows a comparison between the group that received 6 lots of commercial vaccine and the group that received the larger dose of Vaccine A. There is a much better response in those who received the commercial vaccine than those who received small doses of Reference Vaccine A. The Type 2 levels were about equally high in all groups and the Type 3 showed a somewhat lower level.

It is clear from these data that a measurable level of antibody does persist and that the degree of persistence of this state is influenced by the amount of antigen employed.

But what degree of immunity are we seeking to establish? Do we wish to have a persistently demonstrable level of antibody or merely an effective hyperreactive immunologic mechanism? Let us consider such individuals as those in whom antibody levels were once induced (as with the small doses of Reference Vaccine A shown in the upper right hand frame of Fig 40) and in whom antibody is no longer demonstrable 1 year after the booster dose.

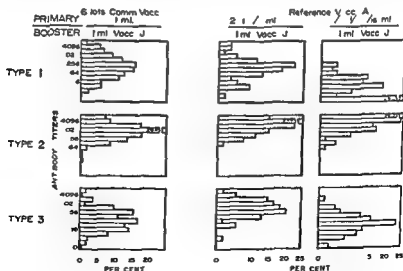


FIG 40 Influence of primary immunization on persistence of antibody. Comparison of commercial vaccines with different doses of Reference Vaccine A.

As an illustrative example Figure 41 indicates the degree of hyperreactivity demonstrable 7 months after primary immunization in one individual and about 1½ years after the last dose of vaccine in another. The level to which antibody increases after inoculation in such individuals suggests the probability that there is an acceleration in the appearance of high levels of antibody. And this brings to mind MacLeod's explanation for long lasting immunity to certain infectious diseases. This is illustrated in Figure 42 and many of you have seen this in MacLeod's paper and in some of our publications.

As applied to polio the expectation is that it is a disease with a long incubation period and therefore the secondary response comes in before the disease process would ensue. As applied to polio in immunologically experienced persons a sufficiently rapid output of antibody in the interval between inception of infection and invasion of or spread within the central nervous system (CNS) could provide the basis for a state of immunity that might be expected to last as long as the hyperreactive state exists. Evidence has already been deduced suggesting that such a mechanism may be operative in preventing the occurrence of Type 1 paralytic polio at least in some persons who have had a prior Type 2 infection.

In such persons there is evidence of a heightened state of immunologic hyperreactivity to Type 1 virus. This is shown in the second column of Figure 43 where you see a group of

individuals who possess no antibody to Type 1 to begin with but do have antibody to Type 2 and they react to the Type 1 component of the vaccine more actively than do those who have no antibody to any of the 3 types. They did not react as actively as those who have had a prior Type 1 infection. This heightened state of immunologic hyperreactivity conceivably could be the critical factor in determining whether or not paralysis will ensue and will determine the degree of paralysis that might develop even though at the time of later exposure to Type 1 infection there is no demonstrable Type 1 antibody in the serum.

The development of such cross immunity reaction seems to be due to the presence of small quantities of Type 1 antigenic groupings which are known to be present in naturally occurring Type 2 viruses. It is remarkable that so small an amount of Type 1 cross relationship as seems to exist in certain strains of Type 2 virus is sufficient to bring about so significant a degree of alteration in the state of immunity to Type 1 paralysis. A similar relationship between the Type 3 and the Type 1 virus is not prominent. If the mechanism suggested is operative in the prevention of paralysis then it is of interest to know whether or not the hyperreactive state resulting from infection or from the injection of a killed virus vaccine is equally effective in the prevention of paralysis.

The importance of this question arises from the fact that it is possible with a killed virus

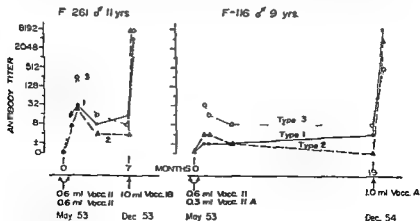


FIG 41 Immunologic hyperreactivity at 7 months and 19 months after primary vaccination



called on to assess the actual production and testing records of many commercial scale lots of vaccine produced by six manufacturers ■ has had the exceptional opportunity of contrasting theories of the inactivation process with practical results. Contrary to published statements the Committee has never taken a position that any one theoretical function or inactivation curve dealing with the loss of infectivity of polio virus under formaldehyde treatment was proved by the results of inactivation experience with commercial scale lots of vaccine. The theoretical considerations are of great importance are controversial in some details and require more investigation but such investigations must be carried out with experimental lots much smaller than those which the Committee was called on to assess.

However to the knowledge of the Committee inactivation data of manufacturers throughout the world show beyond a doubt that the inactivation rate of polio virus as established by Salk is sufficiently approximate to a straight line on a semilog plot so that for practical purposes the time of inactivation of a pool in which all virus particles are accessible to formaldehyde should be predictable from the slope of the line. Recent workers have presented evidence that the inactivation process is more rapid during the

first few hours after addition of formaldehyde than was realized before. Moreover the exact shape of the formaldehyde inactivation curve of this and other viruses especially after the first few days of inactivation ■ still debated. It must be emphasized however that the differences of opinion do not influence the fact that completely satisfactory inactivation can be obtained by means of the present method of inactivation at 37°C with 1:4,000 formaldehyde.

One of the most important findings which has led to the development of a uniform process of vaccine preparation in the United States is the role of filtration or clarification of virus and vaccine fluids in facilitating the completion of the inactivation process. Very early in the analysis of production records it became evident that there was variation in the regularity with which the producers of poliomyelitis vaccine consistently achieved complete inactivation. Analysis by the Committee made it apparent that there were at least two major differences in the manufacturing processes being employed. One method involved the growth of polio virus for vaccine in cultures of minced kidney tissue and clarified the virus fluids by filtration through Seitz type pads which of course are made of felted cellulose and asbestos fibers. A second method of production involved the growth of

TABLE 23 INFLUENCE OF TYPE OF FILTRATION ON COMPLETION OF FORMALDEHYDE INACTIVATION IN PRIMARY PROCESSING OF SINGLE STRAIN POOLS OF ALL 3 TYPES OF POLIO VIRUS

MATERIAL	FILTRATION METHOD	TOTAL NO OF SINGLE STRAIN POOLS	NO OF POOLS INCOMPLETELY INACTIVATED	PER CENT OF POOLS INCOMPLETELY INACTIVATED
A Production from June 1 1955 to Jan 1 1956	Seitz Glass	94 174	2 16	4.1 9.2
B Production from Feb 1 1956 to Feb 1 1957	Seitz Glass	501 230	1 53	0.21 23.0
C Single Strain Pools from Trypsinized Cultures June 1 1955 to Jan 1 1956	Seitz Glass	5 172	0 16	0.0 9.3
D Single Strain Pools from Trypsinized Cultures Feb 1 1956 to Feb 1 1957	Seitz Glass	212 230	1 53	0.47 23.0

A and B include virus produced in Modified type and trypsinized type cultures

■ and D include virus produced in trypsinized type cultures only

virus for vaccine in trypsinized suspensions of monkey kidney cells and the clarification of virus fluids by filtration through fritted glass filters of graded porosities. Fritted glass filters were being used because of experience suggesting that Seitz type filtration of virus grown in trypsinized cultures resulted in serious loss of virus titers. The two methods yielded different results in the uniformity with which inactivation was achieved. This is evident from the findings which I shall show in the following table (Table 23). In A and B one can see that the success of inactivation of material filtered through Seitz type filters in this production period was significantly greater than that with glass filtration, namely 2 per cent of 94 versus 9 per cent of 174 single strain pools. In a later production period an even greater difference is shown with a very successful or satisfactory effect of inactivation with Seitz filtered material.

C and D in Table 23 show material uniformly processed from trypsinized cultures. Here you see that the difference is still apparent and that the difference in favor of the Seitz filter must be attributed to the filtration and not to the method of preparation of the virus fluid. The total experience vividly demonstrates the superiority of the method employing Seitz type filters.

The Committee recommended that further experimental work be done; and the results made available the Committee may be summarized as follows:

1. Seitz type filtration of the virus fluids during the formaldehyde inactivation process was much superior to filtration through fritted glass in yielding satisfactory vaccine. In one laboratory 16 out of 46 glass filtered single strain lots of poliomyelitis virus failed to be completely inactivated satisfactorily by formaldehyde. These experimental lots of virus were produced on a production scale during approximately the same period of time.

2. Untreated polio virus grown in trypsinized culture can be satisfactorily filtered through Seitz type filter pads under proper conditions without significant loss of infectious titer. This was not previously realized.

3. Vaccine prepared from polio virus grown either in trypsinized or Mantland type cultures and clarified before and during the formaldehyde inactivation process by filtration through

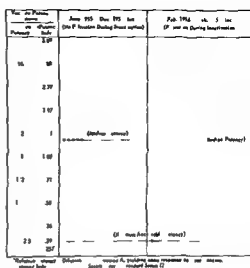


FIG. 44. Type 1. Frequency distribution of potencies of released vaccine lots (all manufacturers) by filtration procedures.)

Seitz type pads possesses satisfactory antigenic potency. However, after the introduction of the double Seitz filtration—that is, before and during the inactivation process—the distribution of potency values of released vaccine lots was somewhat inferior to that obtained when single filtration through either glass or Seitz type filters was employed. The depression of potency from this and probably other sources affects the Type 3 components especially. The next 3 illustrations will show this effect.

With the Type 1 (Fig. 44) these are the potency indices in relation to a standard reference serum. We see that the median potency of released vaccine lots during this period with no filtration during the inactivation process—filtration only prior—is higher than that of lots with the added filtration during inactivation.

Type 2 shows a similar effect (Fig. 45). Type 3 shows a much more pronounced effect which may be attributable not only to the second filtration but also possibly to other factors as well (Figs. 46 A and B).

With the completion of experimental work indicating that pools of Seitz filtered polio virus were inactivated by formaldehyde with much greater regularity than those clarified by filtration through fritted glass and with the further establishment of the fact that antigenic potency

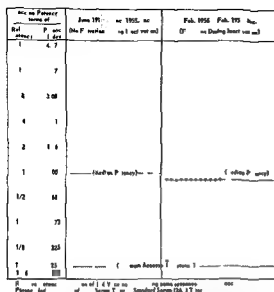


FIG 45 Type 2 Frequency distribution of potencies of released vaccine lots (all manufacturers by filtration procedures)

was retained after Seitz type filtration the Committee recommended modification of the procedure for the manufacture of vaccine (Figs 47 and 48)

It seems evident from the results of these steps that the critical difference in the manufacturing processes was not the method used in producing virus as much as differing filtration practices. This fact leads to the new and important observation that in practice the completion of inactivation of virus fluids depends on carefully controlled filtration procedures. The extraordinarily high degree of homogeneity required for inactivation of the last few virus particles has not been previously recognized as a highly critical condition nor as a difficult achievement but it is probable that failure to achieve it resulted not only in serious difficulties in 1955 with some batches of polio vaccine but also with experimental batches of formalinized vaccine for Venezuelan equine encephalitis.

In retrospect it appears that the advantages of preparing virus from monolayers of monkey kidney cells were unexpectedly outweighed by the more successful filterability of fluids from minced kidney cultures when Seitz pads were used. Of course it is possible that still better methods may be devised for obtaining more efficient filtration of both types of fluids. A better

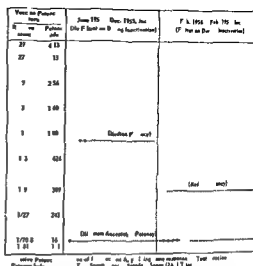


FIG 46A Type 3 Frequency distribution of potencies of released vaccine lots (all manufacturers by filtration procedures)

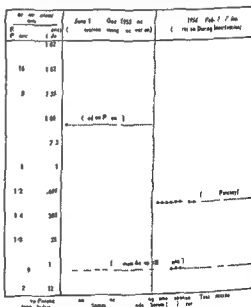


FIG 46B Type 3 Frequency distribution of potencies of released vaccine lots (all manufacturers by filtration procedures)

understanding and control of the intimate details of formaldehyde inactivation could in time enable a closer approach to the dual ideal of complete inactivation plus total retention of antigenicity. Fundamental research in this field still seems to be desirable and the results could

make possible great improvements in the production of many viral antigens.

In relation to safety testing one important finding of the testing experience of vaccine was that the examination of vaccine for residual live virus is not comparable with the problem of measuring infective quantities in untreated live virus suspensions. Apart from statistical considerations of adequacy of sampling and of volume of material to be tested it is now clear that testing of vaccine in tissue cultures should be accompanied by rigorous controls of the sensitivity of the tissue-culture system in each laboratory and controls on the effect of the vaccine fluid on the sensitivity of the system. With proper control the tissue-culture system can be demonstrated to be superior in sensitivity to the monkey safety test partly because of the much greater volume of material tested or stable in tissue cultures. Nevertheless the monkey test on a series of successive batches when negative has always indicated a quality of production associated with a safe product.

However the inability of the monkey test to detect extremely low levels of infectivity in vaccine in comparison with large scale tissue culture tests led to experiments designed to increase the susceptibility of monkeys in the test. By a combination of intraspinal and intramuscular inoculation and with a variety of enhancing procedures two research laboratories obtained many more infected monkeys with virus isolation than were obtained with simple intracerebral monkey tests on the same lots of vaccine which epidemiologic evidence showed to be infectious. These findings and other evidence show that the monkey test could be strengthened by the use of multiple routes of inoculation and of enhancing injections. Such procedures were incorporated into the current regulations.

Although it is certain that the tissue-culture test at its best is more sensitive than any monkey test now available the tissue-culture test may fluctuate in sensitivity to a greater degree in actual practice or may be less sensitive than the monkey in the detection of protected particles of virus which escape inactivation. Current methods of production and testing since October 20 1955 have yielded with one possible exception more than 230 successive trivalent lots which were noninfectious in both the new

monkey safety tests and in tissue-culture tests. Most of these pools contained as much as 500 to 1 000 liters of vaccine each.

The available evidence on current testing practices as well as the original ones supports the earlier expressed conclusion of the Committee that the significance of a negative test for infective virus on a given lot of vaccine is greatly enhanced if the particular lot is one of a series in which tests for infective virus are negative.

One of the incidental findings in connection with the program of safety testing of vaccine in industry and one which has great interest in relation to production of any vaccines using viruses from animal or human sources is that many viruses were found to be latent in asymptomatic monkeys used for production of kidney tissue cultures. At least 28 distinct agents have now been identified which are either occasional contaminants of tissue cultures of monkey tissues or are present in the living animal and are activated in the process of cultivation of kidney cells. These agents produce cytopathogenic effects in tissue cultures. All of them are inactivated by formaldehyde at least as readily as polio virus and therefore present a troublesome problem of identification rather than a threat to safety of the vaccine.

Pre-existing virus infections in monkeys used in the monkey safety testing have also been troublesome since the monkey safety test requires histopathologic confirmation that asymptomatic test animals have not been infected by the vaccine. Analysis of over 100 asymptomatic monkeys with inflammatory or glial lesions of the central nervous system indicates that a small proportion of test animals exhibit lesions of the viral type ranging from obviously old and healed lesions to extensive involvement in the subacute stage. Five categories of lesion distribution which were considered as atypical for poliomyelitis infection have been identified. All were represented by instances in which lesions were so old as clearly to antedate the time of injection. The need for reinforcement of the histopathologic criteria for poliomyelitis infection in doubtful asymptomatic cases has led to the recommendation that final diagnosis in such cases be based on attempts at virus recovery as well as histopathologic evidence. This requirement subsequently led to the interesting finding that at least some of the atypical pathologic

TABLE 24 SURVEY OF TRIVALENT POOLS IN MONKEYS AND IN TISSUE CULTURE (5 INDUSTRIAL LABORATORIES)\*

SURVEY PERIODS	POOLS POSITIVE IN MONKEYS		ALL POOLS		POOLS POSITIVE IN TISSUE CULTURE			POOLS POSITIVE IN BOTH TYPE	
	NO POOLS	PER CENT	NO †	PER CENT	TYPE 1	TYPE 2	TYPE 3	POOLS POSITIVE IN BOTH TYPE	POOLS POSITIVE IN BOTH TYPE
Prefield Trial and Field Trial Periods	53	9.4	2	3.8	1	0	1	2	1
From Field Trial Period to May 26 1955	146	0.7	17	12.0	6	6	11	0	0
From May 26 to Oct 20 1955	62	0.0	7‡	11.0	4	4	2	0	0
From Oct 20 1955 to April 1 1956	107§	0.0	0	0.0	0	0	0	0	0
From April 1 1956 to Oct 1 1957	128		1*	0.78	0	1	0	0	0

\*For reasons beyond the control of the commission data for these surveys are not available from all 6 industrial laboratories. The negative pools which were derived from the experience of the 5 industrial laboratories are correlated with a field appearance which appears to be entirely safe with the possible exception of 1 vaccine lot from the second period.

†Includes pools from which more than 1 type was isolated.

‡Includes 4 pools from a single laboratory. Remaining laboratory had a rate of 5.6 per cent positive if lent pool is in this period.

§The cell is actually in subject d 209 separate monkey. Frequency is in a total of 4,850 monkey because of duplicate tests by either the manufacturer or by the NIH. The laboratory is one of these lots. However, the field test sample is often greatly in excess of requirement.

¶The lot was considered as positive although it could not be established conclusively that the virus isolated was derived from the same lot rather than from common contamination of the culture used in the test.

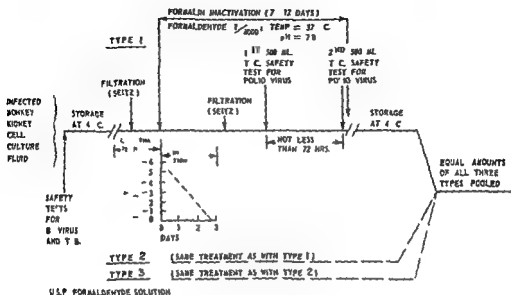


Fig. 4 Single strain pool

pictures observed in test animals were associated with the recovery of the B virus of Sabin and Wright. The abortive nature of the infection of the central nervous system in such asymptomatic animals despite the treatment with cortisone which was administered at the time of inoculation suggests that this virus may be activated by the trauma of the injection into the nervous system.

In the monkey potency test the antibody response elicited in simians by injection of the undiluted vaccine being tested is compared with

that previously elicited by 3 different dilutions of standard vaccine. In preparing the antisera against the standard trivalent vaccine large groups of monkeys were immunized with undiluted 1:4 and 1:16 dilutions of a reference vaccine material from the 3 pools of antiserum were subsequently employed for the comparison with the antiserum produced with each batch of undiluted vaccine being tested. This test served an invaluable function during the first few years of vaccine production. Its results have correlated reasonably well with efficacy of the vaccine.

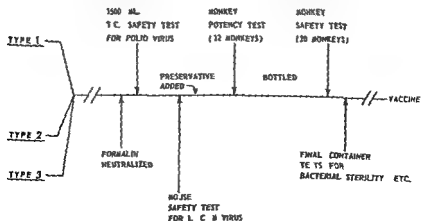


Fig. 48 Trivalent

in human populations as far as limited data show. Its deficiencies have been recognized wherever it has been used in large scale production. A test using small animals and capable of greater precision through the use of larger numbers of animals offers practical advantages and many European laboratories soon adopted the guinea pig test developed by Gard in Sweden. In this test an antigenic extinction titer is obtained by vaccinating groups of animals with successive dilutions of vaccine. The lowest concentration producing an antibody response represents the level of antigenic extinction and is a measure of the potency of the vaccine. A similar test in chicks has been reported and is being developed in an extensive co-operative study by the United States manufacturers and the United States Public Health Service. Early results appear promising.

With such tests in small mammals or chicks a statistically adequate number of animals can be used with considerable economy of effort, animals and antigen. The use of a live virus standard is also possible and affords an ideal target for improvement of potency since the target is the ultimate level achievable by a live nonmultiplying viral antigen. Finally in a single completely controlled test in a single batch of animals the standard antigen can easily be compared with a series of test samples whether these are inactivated by a single method for routine production or are part of an experimental series designed to study different methods of inactivation and their effects on potency.

It is now clear that considerable variation in the potency of different batches of vaccine still exists. The improvement of the means for controlling potency of the vaccine may seem to be a technical detail but on this detail rests the most important remaining problem of vaccine production, namely improving the potency and the uniformity of potency of the vaccine. The development of a convenient and accurate method of potency testing will be an important aid in achieving this objective.

After the unfortunate incident in the spring of 1955 in which the Mahoney Type 1 strain was clearly shown to be virulent in human beings as it is in monkeys a considerable effort was made to find a less virulent strain with equal or better immunizing capacity as well as with the necessary characteristics of stability

during the manufacturing process. The problem has been more difficult than was anticipated. Although strains of relatively low virulence are available no proposed substitute strain which has shown properties during large scale manufacture of vaccine which indicate a clear superiority over the Mahoney strain in antigenic potency in human beings. Obviously the search must continue to find a Type 1 substitute as superior in potency to the Mahoney strain as is the Type 2 component in the vaccine which approaches the antigenic potency of an inactivated virus. The vaccine could also be improved by means of a more stable Type 3 component and there is little doubt that an inactivated vaccine composed of 3 strains equal to the Type 2 component would be greatly superior to any vaccine now being produced.

Looking back at the difficulties encountered in the large scale manufacture of formalinized polio vaccine one can see that much of lasting value has been learned in the aftermath. Pathways to improvement of the vaccine and of its control have been marked out. Most of all the facts and real achievements which were recognized at the last Conference and after the Field Trial of 1954 have stood the test of time of temporary confusion and of controversy. The result is a widely used and widely accepted preparation of demonstrated safety which is of high protective value against paralytic poliomyelitis.

The vaccine discussed here is the one which has been most extensively tested for its preventive power against paralytic poliomyelitis.

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## *Results Obtained by Means of Vaccine Composed of Inactivated Viruses*

DR ALEXANDER D LANGMUIR

In April 1955 when Dr Francis reported on the success of the field trials of inactivated polio virus vaccine a comprehensive mass immunization program was initiated in the United States and has continued to the present time. The objective is to achieve universal immunization of the 109 000 000 persons under 40 years of age in our population. Many problems have been encountered. Some were anticipated and planned for others were unforeseen and had to be met on an emergency basis. Up to April 1957 approximately 145 000 000 doses of vaccine were distributed. A little over half of the goal has been achieved for our population under 20 years of age. Immunization of the adults from 20 to 40 has only barely begun and will not be pushed until more adequate supplies of vaccine become available to us. Other nations notably Denmark have made far greater progress and their experience should be studied with care. But it is also believed that our experience is of some interest from the sheer magnitude of its scope and from the extent of the evaluation which has been attempted. The present paper will summarize the field epidemiologic aspects during 1955 and 1956 with particular emphasis on the evaluation of the safety and the effectiveness of the vaccine and of its use under epidemic situations.

The information to be presented is a summary of the voluminous reports that have been collected in the Poliomyelitis Surveillance Program. This is a major national co-operative effort which includes all states more than 40 virus laboratories many other co-operating organizations and more than 40 epidemic intelligence service officers who have been detailed for full time field duty or at least alerted for first priority duty in any field activity relating to poliomyelitis. This program is continuing at full scale. During its early stages primary concern was directed to investigation of the Cutter

incident. Later emphasis was placed on the evaluation of the effectiveness of the vaccine but at all times a constant surveillance has been maintained on safety of the vaccine actually distributed and used.

### SAFETY OF THE VACCINE

There were 3 distinguishing epidemiologic characteristics of the Cutter cases. First excessive frequency of cases in association with the use of certain particular lots of vaccine this frequency being independent of the prevalence of naturally occurring poliomyelitis in the area. Second a concentration in the interval between inoculation and onset within the period of 4 to 11 days for the vaccinated cases and 8 to 27 days for the contact cases—this 8 to 22 days being essentially a double incubation period. Third correlation between the site of the inoculation and the site of the first paralysis. Now these 3 crucial features were essentially similar to those described for experimental inoculation poliomyelitis in cynomolgus monkeys by Bodian.

The continuing surveillance for the safety of poliomyelitis vaccine in the United States has been based on constant search for these distinguishing characteristics. It was anticipated that as vaccine became more widely used purely coincidental cases of polio among vaccinated persons would be reported with increasing frequency. This of course has occurred.

In the calendar year of 1956 reports were received on 279 cases of paralytic polio which occurred in individuals who had received polio vaccine within 30 days prior to onset. This total does not include a group of more than 300 similar cases from the Chicago epidemic which will be discussed later and separately.

These 279 cases occurred in the large population that had received over 70 000 000 doses of vaccine during the period thus giving a ratio of about 1 paralytic case to every 300 000 inocu-

are of particular interest. They were not associated in excessive frequency with any specific lots of vaccine but on the contrary tended to concentrate in areas where poliomyelitis was prevalent. There was no concentration of the intervals between inoculation and onset in the 4 to 11-day period. Paralysis occurred more than twice as frequently in the legs as in the arms which was a common site for inoculation. While there are obvious limits to this type of epidemiologic evaluation and while it must be recognized that the concept of absolute vaccine safety is not scientifically tenable it is possible to conclude that the frequency of any hypothetical untoward effects resulting in the occurrence of poliomyelitis was less than 1 per million inoculations. Thus it is clear that the more than 100,000,000 doses of vaccine distributed in the United States since the Cutter incident have been safe.

#### NONSPECIFIC REACTIONS FOLLOWING INOCULATION

As part of the surveillance of vaccine safety data have been collected on vaccine reactions. Among those reactions considered to present a possible hazard were encephalitis or other neurologic illness such as may occur following use of smallpox or other vaccines, allergic reactions to the traces of penicillin and other foreign proteins in the vaccine, nephritis or other renal disease attributable to residual monkey kidney proteins in the vaccine, and finally sensitization to Rh negative persons to antigen potentially present in the vaccine.

To evaluate these problems the Poliomyelitis Surveillance Program has collected reports of a number of nonpolio illnesses occurring shortly after vaccination. On the basis of reports that have been received it is evident that vaccine reactions of all types both mild and severe have been rare. It is apparent that poliomyelitis vaccine is associated with at least as low a frequency of reactions as any other immunizing agent in common use today.

#### VACCINE EFFECTIVENESS

During the nationwide campaign for mass vaccination it has not been possible to conduct carefully controlled studies to evaluate effectiveness of the vaccines in actual use. Instead eval-

uation has depended on more qualitative types of studies and on orderly epidemiologic inference based on careful observation and analysis. The consistency with which many different independent studies have indicated a substantial effectiveness against paralytic cases is impressive. The following types of study will be summarized: the total incidence in the nation and the age distribution of cases; the occurrence of paralysis in relation to prior vaccination studies in one particular state, California; and then careful studies of epidemic situations that we have faced.

The annual incidence rates of total reported cases—this includes both paralytic and nonparalytic cases which is customary in the United States—from 1910 to 1956 are shown in Figure 49. The weekly incidence for the 11 years from 1946 to 1956 is shown in Figure 50. A total of 15,000 cases were reported in 1956 as compared with 78,000 cases in 1955. It is necessary to go back to 1947 to find a year with lower total reported incidence. While the widespread use of vaccine in 1956 undoubtedly has influenced this incidence, the experience of several more years will be necessary before the broad effect of mass vaccination can be judged adequately. In our own group we tentatively set 1947 as the low mark beyond which we must go before we can draw confident conclusions from these types of crude epidemiologic data.

During 1955, 33 states co-operated with the Polio Surveillance Program in reporting the paralytic status and the vaccination history of reported cases of polio. During 1956 these 33 states and 12 others joined in this co-operative effort. During the spring and the summer of 1955 the utilization of polio vaccine was limited to the first and second grade school children because of the short supply. As a result a high proportion of 7 and 8 year-old children received at least 1 dose of vaccine before the peak of the 1955 season. The age specific attack rates for reported paralytic cases were significantly lower than in the younger age groups or in the older children through age 14 as is shown in Figure 51.

Note particularly the dotted line which shows the 1955 incidence; there is a notch or a defect in the curve out of character with all previous years experience which relates to the 2 years of age that had received polio vaccine during the

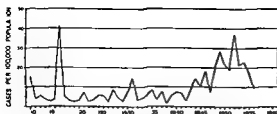


FIG 49 Annual poliomyelitis incidence rate in the US 1910-1956 (U.S. Public Health Service)

NFIP supported clinics in 1955. These lower rates in the 7 and 8 year-old children were interpreted to reflect a higher degree of immunity in these groups resulting from the vaccination.

Now in 1956 vaccine became increasingly available. During the winter and the early spring priority was given in most areas to the 5 to 9 year age group but by April the priority was extended to the age span from 0 to 15 years of age and to pregnant women. By late summer vaccine became available for all under 20 years

of age. Specific figures of the proportion of the population immunized by individual age groups are not available for the nation as a whole. However it can be conservatively estimated that by September 1—essentially the peak of our polio season—approximately 50 per cent of the whole group from 1 to 19 years of age had received one or more doses of vaccine.

Therefore the age specific attack rates for 1956 are of particular interest. You will note that for 1956 the solid curve is shifted sharply to the left (Fig. 51). The peak incidence occurred in the 1 year age group instead of in the 2 to 5 year group as in 1955. The rate in 1956 fell sharply and steadily from the high in 1 year-olds to a low in 9 year-olds after which it rose slightly and remained level through age 30. The low rates in 1956 in the 8 and 9 year-olds correspond to the low rates in the 7 and 8 year-olds of 1955. Since these age groups represent the same children who received first priority vaccination in 1955 the continuing low rates point to a duration of effectiveness of vaccine for at least 1 year.

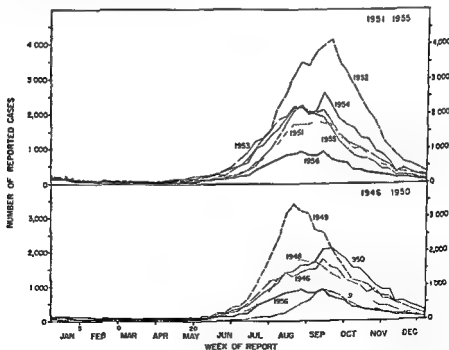


FIG 50 Incidence of poliomyelitis in 1956 as compared with years 1946-1955 (U.S. Public Health Service)

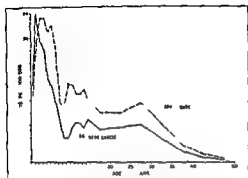


FIG 51 The age distribution of paralytic poliomyelitis in 1955 and 1956 (data from 33 states July-October 1955 and calendar year 1956) (U S Public Health Service)

#### HISTORY OF PARALYSIS IN RELATIONSHIP TO HISTORY OF VACCINATION

During 1956 57 per cent of the total cases of poliomyelitis were classified as paralytic according to the records submitted in the Age Distribution Study. This over all figure is comparable with similar types of data reported in 1955 and again in 1957. When cases reported in 1956 in the under 20 year age group are separated by vaccination history it is found that only 34 per cent of vaccinated cases were paralytic whereas 60 per cent of unvaccinated cases were reported as paralytic. This difference indicates that the vaccination prevented some paralytic cases.

In a study of only the paralytic cases occurring during the midsummer season in the under 20 year group 21 per cent had a history of vaccination. This figure may be compared with the previously mentioned estimate that approximately 50 per cent of this age group had received 1 or more doses of vaccine by midseason. This difference points to a vaccine effectiveness of the order of 75 per cent against paralytic polio.

In the Age Distribution Analysis the co-operating states were not asked to report on the number of doses of vaccine that each vaccinated case had received. A special study of hospitalized cases was performed independently by the National Foundation for Infantile Paralysis which did collect data on numbers of doses of vaccine received. Reports were received on 3 198

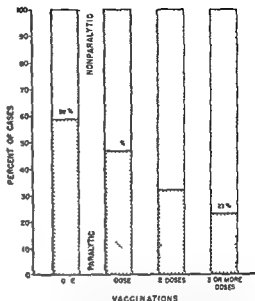


FIG 52 Frequency of paralysis in polio myelitis patients by vaccination history August 15 September 30 1956 (data from a hospital survey by the NFIP) (U S Public Health Service)

acute admissions for polio in 408 hospitals in 48 states. The results are shown in Figure 52. When the cases are grouped according to the number of vaccine doses received prior to onset the frequency of paralysis declines progressively from 59 per cent among unvaccinated cases—which agrees so closely with our figure of 60 per cent—to 23 per cent among those who had received 3 doses. This study not only supports the effectiveness of the vaccine in reducing paralysis but also emphasizes the importance of the full course of 3 doses.

#### STUDIES IN PARTICULAR STATES

During 1956 special studies were more difficult to conduct because of the lack of precise data on utilization of vaccine in specific age and geographic groups. In the state of California however a very intensive effort was made to follow the vaccine distribution month by month and thus the effectiveness could be measured with great care. This study recently released for our general information maintains an effectiveness of well above 75 per cent for those who had received more than 1 dose.

## EPIDEMIC STUDIES

In 1955 a severe epidemic of polio struck in Boston and extended over the entire eastern half of the state of Massachusetts. An extensive study of this epidemic was made by Pope Feemster and co-workers and others. Special attention was directed to the effectiveness of vaccine in first and second grade school children. A school census in that state provided accurate data on populations exposed to infection in various age groups.

The attack rates were high not only in the crowded areas of the city of Boston where the epidemic appeared to originate but they also remained high in the suburban areas and in the smaller communities around Boston. There was no apparent concentration of cases in particular socioeconomic racial or geographic groups. Such almost uniform distribution of disease over

a wide area is characteristic of most large scale epidemics of polio in the United States.

This study in Boston permitted a large scale test of the effectiveness of 1 dose of vaccine under severe epidemic conditions. The results showed an overall effectiveness of 53 per cent against all types of disease including nonparalytic cases and an effectiveness against paralytic cases of 60 per cent. Effectiveness was found to be equally good for 6- and 7-year-old children as well as for 8- and 9-year-old children thus helping to resolve one of the questions raised in the findings of the Francis Report. Also—and I believe of great importance—the effectiveness of the vaccine was found to be greater against the more severe forms of the disease. It was 69 per cent effective against severe and fatal polio.

In Chicago an epidemic of polio began in

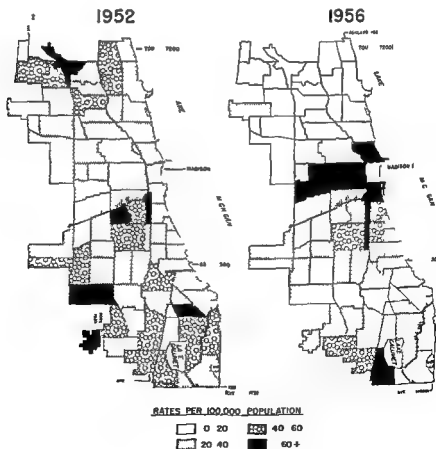


FIG. 53. Poliomyelitis incidence rates in the community areas of Chicago (U.S. Public Health Service)

June 1956. The incidence increased rapidly to a peak in the first week of August and then as rapidly declined. A total of 1106 cases was reported. During the last previous polio epidemic in that city which occurred in 1937 a similar number of cases was reported, namely 1203.

Despite the similarity in incidence that is in total cases during these 2 epidemics there was a striking contrast in other epidemiologic characteristics. In 1952 the cases were widely distributed throughout all areas of the city as is shown in Figure 53. In only a few of the 73 community areas was the rate lower than 20 per 100,000. The disease appeared to attack negroes and whites in all socioeconomic groups in the essentially uniform pattern that is characteristic of large poliomyelitis epidemics. Note on the left hand side of the illustration that in the 1957 distribution of cases throughout the northern part of the city which is a middle and upper-class area there was heavy polio. There were scattered areas here with high and low rates and again high rates in parts of the southern part of the city of middle-class population. In 1956 this northern area was almost wholly free of disease, large parts of the south were free and there was great concentration in the middle range and down in the southern tip which characteristically are now occupied by negroes.

In contrast the cases during 1956 were sharply concentrated in the community areas known to have the lowest socioeconomic status and the highest proportion of negroes. In these areas little opportunity has been provided for poliomyelitis vaccine, particularly for preschool children. *Large areas in the north and the south sides of the city were spared.* These areas house the middle and the upper socioeconomic groups. These groups had greater opportunity to receive polio vaccine through their private physicians.

Other changes in the epidemiologic pattern occurred in 1956. There was a marked shift in incidence to younger age groups, 39 per cent of the white cases and 69 per cent of the nonwhite cases being under 5 years of age in 1956 as compared with 26 and 46 per cent respectively in 1952. Thus the epidemic in Chicago in 1956 presented unique features. In spite of wide spread seeding of the virus throughout the city epidemic rates developed only in a few areas and in the specific age and racial groups con-

taining small numbers of vaccinated persons.

Many authorities have expressed fear that the administration of polio vaccine during an epidemic might result in localization of paralysis due to the so-called provoking effect known to occur following other types of inoculations. Hence in 1955 in Massachusetts in Wisconsin and other areas vaccination was stopped as soon as the existence of epidemics was realized. Therefore no opportunity was presented to test whether or not this fear of the provoking effect was real or to measure the quantitative importance of it.

In October 1955 an epidemic of polio broke out among naval dependents in Pearl Harbor, Hawaii. In the face of a rising incidence of the disease vaccination was given to over 20,000 individuals comprising 80 per cent of the population at the Naval Base. Careful studies by Poos and Nathanson failed to demonstrate evidence of a provoking effect.

This one study cast doubt on the existence or at least on the importance of this hypothetical hazard. In 1956 when epidemic polio broke out in Chicago mass immunization was conducted during the period of the peak incidence. Particular attention was directed to the areas in the city experiencing the highest rates. During a 6 week period from July 20 to September 1, 1,750,000 doses of vaccine were administered. Special studies were conducted by a team from the Communicable Disease Center. Over 1,100 cases of polio were reported during this year (1956). Of these about 300 had a history of recent vaccination of which 75 per cent were paralytic. Among these first paralysis developed in the legs twice as often as in the arms. In those with arm paralysis the sites of inoculation were approximately equally distributed to the paralyzed and the unparalyzed sides. There was no evidence of concentration in an interval of 4 to 11 days. Thus there was no evidence found of a provoking effect. It is concluded that this hypothetical hazard is not of sufficient consequence to inhibit mass vaccination in epidemic situations.

The usefulness of mass vaccination in the face of a rising incidence or an epidemic prevalence of polio requires further evaluation. In the Hawaiian epidemic the incidence dropped sharply about 4 weeks after the mass vaccination began. Later cases were limited largely to

new arrivals. While this experience is suggestive it cannot be considered as conclusive evidence that mass vaccination was responsible for the termination of the epidemic.

In Chicago the mass vaccination began intensively in the last week in July. The peak incidence occurred during the week ending August 3. Such an early peak is most unusual in Chicago, the first of September being the date of expected maximum incidence. However, the Chicago epidemic began earlier in 1956 than in other epidemic years, and the peak occurred sooner than could be expected to have resulted from the mass vaccination program itself. Preliminary studies of the epidemic curve failed to show an accelerated decline or other influence that can be ascribed with any assurance to the immunization program. It is probable that the campaign began too late to exert a material influence on the natural course of the epidemic.

### SUMMARY

In the United States experience with vaccine since the Cutter incident shows that the vaccine has been safe and almost free of severe untoward reactions of a nonspecific character.

The effectiveness of the vaccine has been maintained at the level of approximately 75 per cent or better. Most observations have been based on populations where the majority of persons have received only 1 or 2 doses. The effectiveness of a full course of 3 doses of potent vaccine properly spaced has not yet been measured. Such indications as are available point to an effectiveness greater than 75 per cent. The duration of immunity has not yet been determined. Several more years of study both in the field and in the laboratory will be necessary to determine how long immunity lasts and how frequently booster inoculations should be repeated.

Experience with mass vaccination first in Hawaii and then in Chicago has allayed the fear that such a procedure might provoke the localization of paralysis at the site of inoculation. This fear no longer justifies avoiding mass vaccination in areas with high prevalence. While mass vaccination in all future epidemic situations will probably be administratively unavoidable, it is doubtful that a large enough proportion of the susceptibles in the population can be immunized in time to influence the course of

the epidemic. This I would restrict to the sharp epidemics of the temperate zones rather than in areas experiencing a longer and more protracted outbreak. Very likely vaccination would show a measurable effect in the latter epidemic situation. Wise health officials will endeavor to achieve a high level of immunization prior to the time when poliomyelitis epidemics may occur and thus forestall them.

### CONCLUSION

Our experience with inactivated polio virus vaccine has been encouraging. With continuing progress in achieving increasing levels of immunization in all segments of our population under 40 years of age, a steady reduction in the frequency of paralytic cases can be confidently anticipated.

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## DISCUSSION

**Dr von Magnus.** The short time which has elapsed since vaccination started does not allow final conclusions as to duration of immunity. Dr Salk has a series of observations which give him confidence on this point. Much can be said about the problem of duration of immunity. Even live virus vaccines presently used are not regarded as giving a lifelong immunity. Thus for instance an international smallpox vaccination certificate is valid for only 3 years.

The complications to the vaccination in the United States have been few—fewer than could be reasonably expected. Observations in our country were approximately the same as those reported by Dr Langmuir. In our country 7500 000 individuals have been vaccinated intradermally 2 or 3 times by now. Various observations on this vaccination program are presented in the Scientific Exhibits and I will not comment on it here.

As far as safety is concerned I will remind you of a sentence by Dr John R. Paul from his closing remarks at the Conference in 1954. Referring to the future use of a killed polio vaccine he stated: "Accidents are bound to happen." As we all know an accident did happen. The impression on people in all parts of the world was tremendous. But perhaps it can be said and personally I feel it very deeply indeed that this unfortunate accident which happened early at the very beginning of the mass vaccination program in the United States has prevented a number of possibly even more severe accidents in connection with vaccines prepared in other parts of the world. With the careful controlled procedures now in use the product can be said to be safe as stated by Drs Bodian and Langmuir.

The need for polio vaccine is great in many countries and in most places there will not be enough vaccine for the first few years. Even if the United States and maybe other countries at some point in the future may have a real surplus of vaccine economic considerations and currency problems most certainly will be a barrier for the distribution of this surplus to a number of other countries.

Since we in Denmark have used the intradermal route for inoculation of polio vaccine and since less volume of vaccine is used per individual by this mode of injection we have been asked by health authorities in a number of other countries whether we would advise them to use the intradermal route in their own country or not. On an experimental scale Dr Salk has been able to induce antibody formation to all 3 types of virus in 100 per cent of the vaccinated whether the vaccine was given intradermally, subcutaneously or intramuscularly. Others have made similar observations on an experimental scale.

However vaccines prepared on a larger scale and used so far in mass vaccination programs are less antigenic and to my knowledge do not induce antibodies in 100 per cent of those vaccinated. The data available to us at present on vaccination with such vaccines in animals and in humans indicate that 1 ml of vaccine given subcutaneously yields a better protection for an individual than 1/5 or 1/4 of 1 ml given intradermally. However our data also support the observation made by Dr Salk several years ago that one obtains a better utilization of a given amount of vaccine and more people are protected if the intradermal route is used.

**Dr Hill.** In Great Britain we have traveled a slightly different road and the contrast is perhaps of interest. In the British vaccine the Brunenders Type 1 strain replaced the more virulent Mahoney. Early last year it was administered in 2 doses at an interval of 3 to 4 weeks to nearly 150 000 children aged 1 to 9 years. They were chosen randomly according to their month of birth from nearly 2 000 000 children whose parents registered them for vaccination but for all of whom supplies were not available. Subsequent observations showed an incidence of paralytic disease in the vaccinated children only about one fifth of the incidence in the unvaccinated. Thus in 75 000 vaccinated children aged 5 to 9 years 1 case of paralytic poliomyelitis occurred at the attack rate of the unvaccinated children there would have been 6. In 75 000



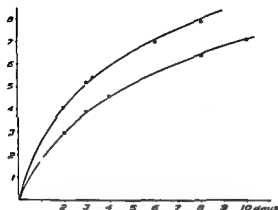


FIG 54 Inactivation of polio virus by formaldehyde Abscissa time in days Ordinate inactivation in log units Curves = equation (Wesslen Lycke Gard and Olin Arch Virusforsch 7 184)

vaccinated younger children aged 1 to 5 there were 3 paralytic cases at the attack rate of the unvaccinated children there would have been 15 These differences are statistically significant to use the magic phrase but the numbers are obviously too few to define accurately the level of protection induced At its face value it is about 80 per cent much the same as was noted in the original American trial And as in that trial the incidence of nonparalytic poliomyelitis appeared to be unchanged

In short the assessment of this British vaccine in the 1956 poliomyelitis season gave two important answers It showed that the milder Brunenders strain conferred protection against Type I infections it showed that protection could be given to the preschool child a point as Dr Langmuir points out not determined in the original American trial

As stressed by Dr Salk two unresolved problems lie in the duration of immunity and in ways and means of increasing the protection given to nearer 100 per cent On the duration of immunity we in Britain cannot add evidence on a strictly controlled basis In fulfillment of a promise we have been busily vaccinating our previous control children that is the registered but unvaccinated children Therefore we can make no further controlled observations Like Dr Langmuir we shall have to fall back upon the inferior evidence of secular trends The

serious difficulties of interpretation were well illustrated in one of his figures

Clearly too it is impossible to use controlled field trials to answer the many questions raised by Dr Salk—the amount of antigen to use the intervals between injections Practice inevitably will have to rest upon evidence of the antigenic stimulus produced However there is one question of fundamental importance—whether 3 doses are substantially more effective than 2 The proportional and scanty figures from the questionnaire mailed to hospitals given by Dr Langmuir clearly cannot carry complete conviction as he himself was most careful to point out At present I know of no other satisfactory statistical evidence and in particular I doubt the figures collected during the Chicago epidemic

But to reach a sound conclusion on that point would need a field trial on a vast scale Suppose the 2 injections reduce a normal attack rate of 20 per 100 000 by three quarters that is to 5 per 100 000 Suppose that 3 injections might further reduce it to 2 per 100 000 that is by 90 per cent, all told To substantiate that change from 5 to 2 would call for a strictly controlled trial involving well over a million children I am not at all sure that such a trial can be made I myself have no doubt whatever of its importance

DR GARD I cannot agree with Dr Bodian on the question of the kinetics of virus inactivation nor on the advisability of repeated filtration in the course of the process At the previous conference in Rome I reported the observations of a Swedish team that the course of the reaction deviated significantly and systematically from that of a first order These studies have been continued and extended mainly by Lycke in our laboratories

We have found that the reaction is adequately described by the formula

$$\log y - \log y_0 = a \log (1 + bt)$$

( $y$  = initial activity  $y$  = activity at time  $t$ )

I direct your attention to the fact that 2 constants are contained in the formula the constants  $a$  and  $b$  We will have an opportunity to mention those later Figure 54 shows the fit of observed data to the theoretical formula—the curves representing the formula

As the formula has been found to apply to a number of viruses tested (not only polio viruses

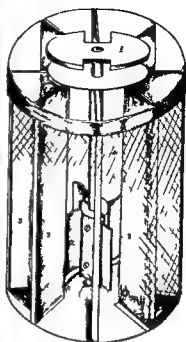
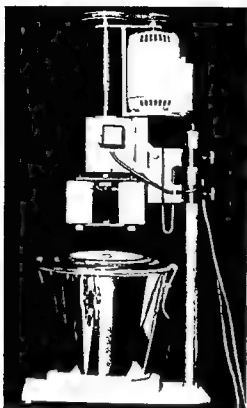


FIG 55 (Left) Homogenizer used in inactivation experiments (Right) Interior of homogenizer

of all 3 types but also Theiler's virus, bacteriophage, influenza virus, foot and mouth disease virus and tobacco mosaic virus) it appeared to be a formula of universal applicability and therefore not to be affected by more or less incidental variations like aggregation or precipitation in the medium. This has been borne out in systematic studies in our laboratory. Thus we have found that filtration, chemical purification, treatment by enzymes and mechanical homogenization have had no effect whatsoever on the course of the reaction. I can show one example.

Lycke has designed a very powerful homogenizer. It is a stainless steel container which is hermetically sealed (Fig 55). Above it you see an electromagnet that drives the movable parts. The interior of this mixer is also shown. The propeller spins at a rate of 600 rpm, passing at a very short distance from a number of stationary lamellas. In addition to the mixing effect of this device, very high velocity gradients are produced each time the propeller blades pass the

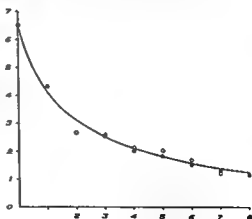


FIG 56 Inactivation by formaldehyde with (o) and without (•) continuous homogenization

lamellas actually 4000 cm/sec/cm at a frequency of 320 times per second.

Figure 56 shows an experiment where two

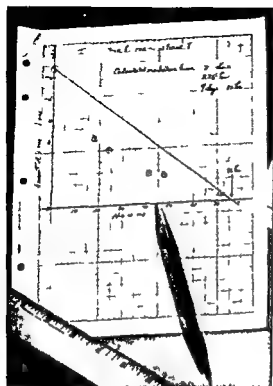
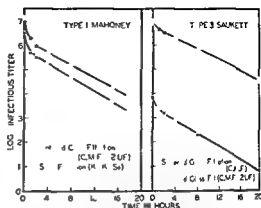
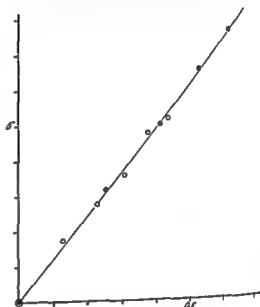


FIG 57 Inactivation curve

identical lots of virus were treated with formaldehyde one under continuous homogenization day and night in the device previously shown the other without any stirring whatsoever. As you can see both curves take exactly the same course.

Dr. Bodian referred to the world wide experience from commercial manufacturers. I have to confine my remarks to what has been made public. First I would like to present an illustration (Fig. 57) showing how to draw a straight line through observed points. Figure 58 likewise shows curved lines. Incidentally it also confirms our experience that filtration has no effect on the shape of the curve; it only retains a certain amount of virus. As you can see the titers were greatly diminished after filtration.

Finally only recently data from a large number of routine batches from the Behringwerke in Germany were published. We have fitted the German results to our theoretical formula (Fig. 59). Now this is a special representation. The 2 constants  $a$  and  $b$  were calculated and  $\log$  inactivation was plotted against the expression

FIG 58 Effect of filtration on inactivation by formaldehyde (Timm, McLean, Kupsky and Hook, *J. Immunol.* 77:444)FIG 59 Inactivation by formaldehyde. Author's observations on 21 vaccine batches = ■ Date of Haas et al. from 60 batches = • Abscissa  $\log(1 + bt)$  Ordinate mean inactivation in log units. Parameters  $a = 12.5$ ,  $b = 0.339$  and  $0.779$  respectively.

$\log(1 + bt)$ . The result should be a straight line if the data fit the formula. As you can see the black points (the German data) fit extremely well and they also agree fairly well with our results (the open circles). On the basis of this evidence I consider intermediate filtration to be not only unnecessary but also harmful because (1)

it removes considerable quantities of virus giving a vaccine of inferior immunizing capacity and (2) it disturbs the regularity of the reaction making the course of the inactivation unpredictable

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Dr SWIDEL I believe that in the manufacturing of polio vaccine we have completed another era in the viral vaccines which in many respects is comparable to that of a decade or so ago when the yellow fever vaccines were first made. What have we learned from polio vaccine that will be incorporated at least in the thinking in any viral vaccines made in the next decade or so? There are 5 points to consider

1 Mass tissue-culture methods can be used successfully for the large scale production of a vaccine for human beings. We can confidently anticipate that many of our viral vaccines of the future will be produced in a similar manner

2 We are aware that commercial vaccines can be made which are relatively rich in viral antigens and poor in nonviral proteinaceous material. Moreover the means are at hand for increasing the former and decreasing the latter

3 The transformation of safety testing of a killed vaccine from an art to a science has been hastened by measures incorporated in the polio vaccine production since the spring of 1955. All future tests on materials made from potentially hazardous agents will be based on more sophisticated statistical concepts than those generally applied earlier. Moreover the quantity of materials

employed in safety tests of new vaccines is unlikely to be much less than the equivalent of those now used in polio procedures. The idea of obtaining and utilizing for safety tests carefully standardized cultures or animals having a high degree of susceptibility to minute amounts of live virus undoubtedly will be adopted in procedures for other viral vaccines

4 The idea is firmly established in the minds of many who have dealt with polio vaccine that greatest dependence can be placed on procedures in commercial production when each naked virus particle is exposed to the killing agent. Aggregates of particles or protective coatings of any type on viral particles may impede inactivation. Seitz filtration which is a required part of the inactivation procedure of the American polio vaccine presumably contributes to consistent inactivation by ensuring that all virus particles subjected to formalin are in an unprotected state. Until an exact understanding is available of the means by which Seitz filtration accomplishes this it is unlikely that polio or other vaccines made in the United States from dangerous viruses of the small or medium size will fail to incorporate a filtration procedure

5 The large scale use of monkey tissue cultures has brought to light a new group of simian viruses some of which are related to previously known agents affecting man. As pointed out by Dr Bodian the problems raised by these viruses have been solved by the manufacturers in the production of polio vaccine and these same solutions no doubt will apply to other killed vaccines made from such starting material. However the simian agents will create additional problems for those who plan to make live attenuated viral vaccines from monkey kidney tissue cultures

Dr Bodian has also touched upon the matter of the Mahoney strain. The idea is a sound one that an avirulent organism is better for making a killed vaccine in case one has an accident than is a virulent one. But as he also pointed out it is as important to have an immunogenic material as it is to have one that is not hazardous

Dr BAZELEY Wide scale immunization against polio was commenced in Australia at the beginning of June 1956. This program was undertaken without preliminary field trials. The wide



TABLE 26 POLIO RELATIVE TO COMMENCEMENT OF VACCINATION IN AUSTRALIA  
[JUNE 1 1956 TO MAY 31 1957]

AMONG 1012000 VACCINATED CHILDREN	EXPECTED	ACTUAL
Polio cases less than 7 days after first dose	8	3
Polio cases between 7 and 28 days after first dose	25	4
Polio cases after second dose at any time during period	400	1
Polio cases for Australia (whole population) 12 month period (July 1 1956 to June 30 1957)	2753	224 (335)†
Polio cases for Australia (whole population) 5 months (January 1 1957 to May 31 1957)	124	71 (105)†

Expected figures based on incidence of polio during the previous year  
† Figures are estimates of incidence of polio during the period of observation

seems clear that the vaccine has markedly lowered the incidence of poliomyelitis during the first 12 months of its use. At no time in the past 12 years has the incidence fallen to such dramatically low levels as since the beginning of vaccination.

The vaccine used in Australia was designed on the basis of experience acquired with Dr Jonas E. Salk at the Virus Research Laboratory, Pittsburgh, U.S.A. throughout its period of development. The preparation and the performance of Reference Vaccine A which originated from his laboratory was the basis for planning the vaccine to be used in Australia. Early work with Vaccine A had showed that the Type 1 component was not equally antigenic with the Type 2 or the Type 3 components in terms of uniform antibody response levels in the human subject and in monkeys. Vaccine A was prepared by mixing equal parts of inactivated single virus type pools similarly prepared otherwise. An adjustment was made in our case whereby a two-fold concentration of the normal Type 1 component relative to Types 2 and 3 was achieved. The vaccine used in Australia thus bears an original live-culture liquid volume relationship of 2:1:1

for each of the three types respectively, all other factors being the same. In this ratio the Type 2 and 3 components are considered to be at least equivalent in strength to the Type 1.

In view of the overall powerful immunizing value of Reference Vaccine A and also the need to produce a vaccine of reasonably small volume for injection purposes, a dose of 0.5 ml. was considered likely to be satisfactory in producing reasonably good immunity. Two intramuscular doses were given in the primary phase 1 month

TABLE 27 COMPARISON OF VACCINE POTENCIES  
VIRUS ASSAY RELATIVE TO THAT OF VACCINE A\*  
MONKEY ANTIBODY PRODUCTION RELATIVE  
TO THAT OF VACCINE A†

VACCINE TYPE BATCH	POTENCY BASED ON VIRUS ASSAY			POTENCY BASED ON SEROLOGY		
	TYPE 1	TYPE 2	TYPE 3	TYPE 1	TYPE 2	TYPE 3
6	1.3	0.8	0.6	1.2	0.6	0.8
7	1.6	0.8	0.8	2.0	0.8	0.8
8	1.7	0.5	0.6	1.5	0.7	1.6
9	2.0	0.6	0.8	1.4	0.8	0.5
10	2.0	0.6	0.8	1.3	0.7	0.7
11	2.5	1.2	0.6	5	0.8	1.1
12	2.0	1.2	0.6	2.2	1.2	1.2
13	1.3	1.0	0.6	1.6	1.3	1.5
14	1.6	1.0	0.6	2.3	1.4	1.3
15	1.6	0.8	0.6	2.5	1.5	1.5
16	1.6	0.6	0.6	2.0	0.9	1.4

\* Virus assay expressed as potency by taking the ratio of the number of virus particles per 0.5 ml. of vaccine to the number of virus particles per 0.5 ml. of Reference Vaccine A. In this case Vaccine A is taken as 1.0. The ratio of the number of virus particles per 0.5 ml. of vaccine to the number of virus particles per 0.5 ml. of Reference Vaccine A is given in the table.

Example	Reference Vaccine A	Batch 6	Batch 6/Vaccine A
Type 1	10 <sup>4.5</sup>	10 <sup>4.7</sup>	1.2
Type 2	10 <sup>4.8</sup>	10 <sup>4.8</sup>	0.8
Type 3	10 <sup>4.9</sup>	10 <sup>4.9</sup>	0.6
* All virus titers related to per cent of NIH Standard 78 (15) (Type 1)			

† Monkey serum potencies were obtained by means of geometric mean of antibody response after graded doses of vaccine given on days 1, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, 105, 112, 119, 126, 133, 140, 147, 154, 161, 168, 175, 182, 189, 196, 203, 210, 217, 224, 231, 238, 245, 252, 259, 266, 273, 280, 287, 294, 301, 308, 315, 322, 329, 336, 343, 350, 357, 364, 371, 378, 385, 392, 399, 406, 413, 420, 427, 434, 441, 448, 455, 462, 469, 476, 483, 490, 497, 504, 511, 518, 525, 532, 539, 546, 553, 560, 567, 574, 581, 588, 595, 602, 609, 616, 623, 630, 637, 644, 651, 658, 665, 672, 679, 686, 693, 700, 707, 714, 721, 728, 735, 742, 749, 756, 763, 770, 777, 784, 791, 798, 805, 812, 819, 826, 833, 840, 847, 854, 861, 868, 875, 882, 889, 896, 903, 910, 917, 924, 931, 938, 945, 952, 959, 966, 973, 980, 987, 994, 1001, 1008, 1015, 1022, 1029, 1036, 1043, 1050, 1057, 1064, 1071, 1078, 1085, 1092, 1099, 1106, 1113, 1120, 1127, 1134, 1141, 1148, 1155, 1162, 1169, 1176, 1183, 1190, 1197, 1204, 1211, 1218, 1225, 1232, 1239, 1246, 1253, 1260, 1267, 1274, 1281, 1288, 1295, 1302, 1309, 1316, 1323, 1330, 1337, 1344, 1351, 1358, 1365, 1372, 1379, 1386, 1393, 1400, 1407, 1414, 1421, 1428, 1435, 1442, 1449, 1456, 1463, 1470, 1477, 1484, 1491, 1498, 1505, 1512, 1519, 1526, 1533, 1540, 1547, 1554, 1561, 1568, 1575, 1582, 1589, 1596, 1603, 1610, 1617, 1624, 1631, 1638, 1645, 1652, 1659, 1666, 1673, 1680, 1687, 1694, 1701, 1708, 1715, 1722, 1729, 1736, 1743, 1750, 1757, 1764, 1771, 1778, 1785, 1792, 1799, 1806, 1813, 1820, 1827, 1834, 1841, 1848, 1855, 1862, 1869, 1876, 1883, 1890, 1897, 1904, 1911, 1918, 1925, 1932, 1939, 1946, 1953, 1960, 1967, 1974, 1981, 1988, 1995, 2002, 2009, 2016, 2023, 2030, 2037, 2044, 2051, 2058, 2065, 2072, 2079, 2086, 2093, 2100, 2107, 2114, 2121, 2128, 2135, 2142, 2149, 2156, 2163, 2170, 2177, 2184, 2191, 2198, 2205, 2212, 2219, 2226, 2233, 2240, 2247, 2254, 2261, 2268, 2275, 2282, 2289, 2296, 2303, 2310, 2317, 2324, 2331, 2338, 2345, 2352, 2359, 2366, 2373, 2380, 2387, 2394, 2401, 2408, 2415, 2422, 2429, 2436, 2443, 2450, 2457, 2464, 2471, 2478, 2485, 2492, 2499, 2506, 2513, 2520, 2527, 2534, 2541, 2548, 2555, 2562, 2569, 2576, 2583, 2590, 2597, 2604, 2611, 2618, 2625, 2632, 2639, 2646, 2653, 2660, 2667, 2674, 2681, 2688, 2695, 2702, 2709, 2716, 2723, 2730, 2737, 2744, 2751, 2758, 2765, 2772, 2779, 2786, 2793, 2800, 2807, 2814, 2821, 2828, 2835, 2842, 2849, 2856, 2863, 2870, 2877, 2884, 2891, 2898, 2905, 2912, 2919, 2926, 2933, 2940, 2947, 2954, 2961, 2968, 2975, 2982, 2989, 2996, 3003, 3010, 3017, 3024, 3031, 3038, 3045, 3052, 3059, 3066, 3073, 3080, 3087, 3094, 3101, 3108, 3115, 3122, 3129, 3136, 3143, 3150, 3157, 3164, 3171, 3178, 3185, 3192, 3199, 3206, 3213, 3220, 3227, 3234, 3241, 3248, 3255, 3262, 3269, 3276, 3283, 3290, 3297, 3304, 3311, 3318, 3325, 3332, 3339, 3346, 3353, 3360, 3367, 3374, 3381, 3388, 3395, 3402, 3409, 3416, 3423, 3430, 3437, 3444, 3451, 3458, 3465, 3472, 3479, 3486, 3493, 3500, 3507, 3514, 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5845, 5852, 5859, 5866, 5873, 5880, 5887, 5894, 5901, 5908, 5915, 5922, 5929, 5936, 5943, 5950, 5957, 5964, 5971, 5978, 5985, 5992, 5999, 6006, 6013, 6020, 6027, 6034, 6041, 6048, 6055, 6062, 6069, 6076, 6083, 6090, 6097, 6104, 6111, 6118, 6125, 6132, 6139, 6146, 6153, 6160, 6167, 6174, 6181, 6188, 6195, 6202, 6209, 6216, 6223, 6230, 6237, 6244, 6251, 6258, 6265, 6272, 6279, 6286, 6293, 6300, 6307, 6314, 6321, 6328, 6335, 6342, 6349, 6356, 6363, 6370, 6377, 6384, 6391, 6398, 6405, 6412, 6419, 6426, 6433, 6440, 6447, 6454, 6461, 6468, 6475, 6482, 6489, 6496, 6503, 6510, 6517, 6524, 6531, 6538, 6545, 6552, 6559, 6566, 6573, 6580, 6587, 6594, 6601, 6608, 6615, 6622, 6629, 6636, 6643, 6650, 6657, 6664, 6671, 6678, 6685, 6692, 6699, 6706, 6713, 6720, 6727, 6734, 6741, 6748, 6755, 6762, 6769, 6776, 6783, 6790, 6797, 6804, 6811, 6818, 6825, 6832, 6839, 6846, 6853, 6860, 6867, 6874, 6881, 6888, 6895, 6902, 6909, 6916, 6923, 6930, 6937, 6944, 6951, 6958, 6965, 6972, 6979, 6986, 6993, 7000, 7007, 7014, 7021, 7028, 7035, 7042, 7049, 7056, 7063, 7070, 7077, 7084, 7091, 7098, 7105, 7112, 7119, 7126, 7133, 7140, 7147, 7154, 7161, 7168, 7175, 7182, 7189, 7196, 7203, 7210, 7217, 7224, 7231, 7238, 7245, 7252, 7259, 7266, 7273, 7280, 7287, 7294, 7301, 7308, 7315, 7322, 7329, 7336, 7343, 7350, 7357, 7364, 7371, 7378, 7385, 7392, 7399, 7406, 7413, 7420, 7427, 7434, 7441, 7448, 7455, 7462, 7469, 7476, 7483, 7490, 7497, 7504, 7511, 7518, 7525, 7532, 7539, 7546, 7553, 7560, 7567, 7574, 7581, 7588, 7595, 7602, 7609, 7616, 7623, 7630, 7637, 7644, 7651, 7658, 7665, 7672, 7679, 7686, 7693, 7700, 7707, 7714, 7721, 7728, 7735, 7742, 7749, 7756, 7763, 7770, 7777, 7784, 7791, 7798, 7805, 7812, 7819, 7826, 7833, 7840, 7847, 7854, 7861, 7868, 7875, 7882, 7889, 7896, 7903, 7910, 7917, 7924, 7931, 7938, 7945, 7952, 7959, 7966, 7973, 7980, 7987, 7994, 8001, 8008, 8015, 8022, 8029, 8036, 8043, 8050, 8057, 8064, 8071, 8078, 8085, 8092, 8099, 8106, 8113, 8120, 8127, 8134, 8141, 8148, 8155, 8162, 8169, 8176, 8183, 8190, 8197, 8204, 8211, 8218, 8225, 8232, 8239, 8246, 8253, 8260, 8267, 8274, 8281, 8288, 8295, 8302, 8309, 8316, 8323, 8330, 8337, 8344, 8351, 8358, 8365, 8372, 8379, 8386, 8393, 8400, 8407, 8414, 8421, 8428, 8435, 8442, 8449, 8456, 8463, 8470, 8477, 8484, 8491, 8498, 8505, 8512, 8519, 8526, 8533, 8540, 8547, 8554, 8561, 8568, 8575, 8582, 8589, 8596, 8603, 8610, 8617, 8624, 8631, 8638, 8645, 8652, 8659, 8666, 8673, 8680, 8687, 8694, 8701, 8708, 8715, 8722, 8729, 8736, 8743, 8750, 8757, 8764, 8771, 8778, 8785, 8792, 8799, 8806, 8813, 8820, 8827, 8834, 8841, 8848, 8855, 8862, 8869, 8876, 8883, 8890, 8897, 8904, 8911, 8918, 8925, 8932, 8939, 8946, 8953, 8960, 8967, 8974, 8981, 8988, 8995, 9002, 9009, 9016, 9023, 9030, 9037, 9044, 9051, 9058, 9065, 9072, 9079, 9086, 9093, 9100, 9107, 9114, 9121, 9128, 9135, 9142, 9149, 9156, 9163, 9170, 9177, 9184, 9191, 9198, 9205, 9212, 9219, 9226, 9233, 9240, 9247, 9254, 9261, 9268, 9275, 9282, 9289, 9296, 9303, 9310, 9317, 9324, 9331, 9338, 9345, 9352, 9359, 9366, 9373, 9380, 9387, 9394, 9401, 9408, 9415, 9422, 9429, 9436, 9443, 9450, 9457, 9464, 9471, 9478, 9485, 9492, 9499, 9506, 9513, 9520, 9527, 9534, 9541, 9548, 9555, 9562, 9569, 9576, 9583, 9590, 9597, 9604, 9611, 9618, 9625, 9632, 9639, 9646, 9653, 9660, 9667, 9674, 9681, 9688, 9695, 9702, 9709, 9716, 9723, 9730, 9737, 9744, 9751, 9758, 9765, 9772, 9779, 9786, 9793, 9800, 9807, 9814, 9821, 9828, 9835, 9842, 9849, 9856, 9863, 9870, 9877, 9884, 9891, 9898, 9905, 9912, 9919, 9926, 9933, 9940, 9947, 9954, 9961, 9968, 9975, 9982, 9989, 9996, 10003, 10010, 10017, 10024, 10031, 10038, 10045, 10052, 10059, 10066, 10073, 10080, 10087, 10094, 10101, 10108, 10115, 10122, 10129, 10136, 10143, 10150, 10157, 10164, 10171, 10178, 10185, 10192, 10199, 10206, 10213, 10220, 10227, 10234, 10241, 10248, 10255, 10262, 10269, 10276, 10283, 10290, 10297, 10304, 10311, 10318, 10325, 10332, 10339, 10346, 10353, 10360, 10367, 10374, 10381, 10388, 10395, 10402, 10409, 10416, 10423, 10430, 10437, 10444, 10451, 10458, 10465, 10472, 10479, 10486, 10493, 10500, 10507, 10514, 10521, 10528, 10535, 10542, 10549, 10556, 10563, 10570, 10577, 10584, 10591, 10598, 10605, 10612, 10619, 10626, 10633, 10640, 10647, 10654, 10661, 10668, 10675, 10682, 10689, 10696, 10703, 10710, 10717, 10724, 10731, 10738, 10745, 10752, 10759, 10766, 10773, 10780, 10787, 10794, 10801, 10808, 10815, 10822, 10829, 10836, 10843, 10850, 10857, 10864, 10871, 10878, 10885, 10892, 10899, 10906, 10913, 10920, 10927, 10934, 10941, 10948, 10955, 10962, 10969, 10976, 10983, 10990, 10997, 11004, 11011, 11018, 11025, 11032, 11039, 11046, 11053, 11060, 11067, 11074, 11081, 11088, 11095, 11102, 11109, 11116, 11123, 11130, 11137, 11144, 11151, 11158, 11165, 11172, 11179, 11186, 11193, 11200, 11207, 11214, 11221, 11228, 11235, 11242, 11249, 11256, 11263, 11270, 11277, 11284, 11291, 11298, 11305, 11312, 11319, 11326, 11333, 11340, 11347, 11354, 11361, 11368, 11375, 11382, 11389, 11396, 11403, 11410, 11417, 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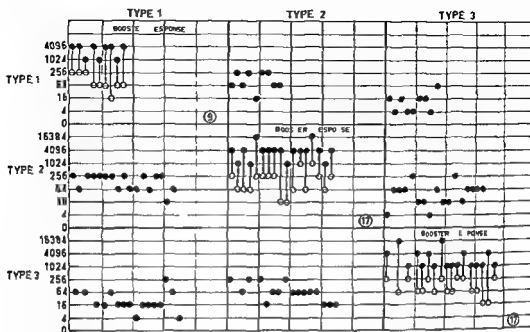


FIG. 62. Antibody response in 43 school children with previous antibody to 1 type only (initial titers determined as in legend for Fig. 61). Two doses 4 weeks apart vaccine batches 1 and 2.

apart with the booster dose to follow approximately 12 months later. The observations with children (recorded in Fig. 61) showed that the composition of the Australian vaccine was capable of stimulating an equal response to all 3 virus types in triple negative antibody children. This dose size also produced a generally high antibody level in a high percentage of subjects.

When the potency of a number of batches of vaccine used in the campaign was examined by means of the monkey antiserum tissue-culture test system it was found that in general the levels established with Reference A had been equaled or surpassed with many consecutive batches of the vaccine. In addition however these potencies appeared to be well related to the original virus concentrations of the vaccine before inactivation (Table 27). It seems reasonable to conclude from this work that a comparative relationship exists between the virus concentration a fluid has before inactivation and its final immunizing potency.

The assay of virus fluids may well prove to be equally reliable or superior to the serologic test for immunizing potency. It will always be difficult to employ and test a sufficient number of

animals to produce serologic values of equal statistical significance to those readily obtained with a sound virus assay. Reliance on virus assay of course is dependent on a constant inactivation procedure which we have always been able to follow i.e. 10 days of inactivation with 1/4000 formalin added the fluids being held at 36°C.

Field response of the vaccine with groups of children has been studied. Using a roller-culture technique for serologic determinations and screening the groups very thoroughly it was found that only 10-12 per cent of some 681 children of all school ages examined could be regarded as completely free from demonstrable antibody in each of the 3 types of polio virus before vaccine doses were given. This low percentage of triple zero antibody children was a surprise.

The bulk of the children thus already possessed 1 or more antibodies. Studying the response in those with a single prior antibody the vaccine dose produced levels that were high or low according to the level of the pre-existing antibody (Fig. 62).

Some indications of the degree of boost provided by a pre-existing heterologous antibody with a subsequent single vaccine dose arose out

TABLE 28 SERUM ANTIBODY RESPONSE IN SCHOOL CHILDREN INTRAMUSCULAR INJECTION OF 0.5 ml OF VACCINES BATCHES 1<sup>2</sup> OR 11 4 WEEKS APART

Geometric Mean Titers In

(a) Triple Negatives

	Type 1	Type 2	Type 3
(b) Double Negatives†	70.65	73.73	19.97
1 Type 1 present		101.6	10.1
2 Type 2 present	133.6		33.4
3 Type 3 present	2.8	64.0	
Booster Response to Type Already Present			
4 Final Response	7580.0	3384.0	7136.0
Pre-existing Titer	87.1	133.6	96.4

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of the above work (Table 28). The mean response achieved was perceptible but not to be compared with the amount of homologous booster response noted. It was particularly pleasing to note the uniformity of the booster response in pre-existing natural homologous antibody together with the high antibody levels achieved. At a later date these will be compared with the nature of the response when attenuated antibody alone is present.

Whether the degree of immunization reached in Australia by the above procedures is needed to control poliomyelitis is of course not clear from the above results. However the program has proceeded in a very satisfactory manner.

We have developed a feeling of some security in this matter by the use at the outset of a moderately concentrated polio virus antigen of fairly uniform composition prepared with constant procedures.

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Dr. GEAR. After preliminary studies initiated in 1957 in collaboration with Dr. Thomas Wel-

ler, who it may be remembered with Professor Enders and Dr. Robbins was responsible for the fundamental discovery that poliovirus would grow in tissue cultures of non-nervous human tissue, production was begun in 1953. The method of production with minor modifications was that prescribed by Salk. We have preferred the Brunenders strain to the Mahoney strain as a representative of Type 1. The formaldehyde material on hand has been supplemented by treatment with ultraviolet light.

As with most others engaged on a similar task, we have had our difficulties. And in solving them we would like to acknowledge the generous help that we have received from our friends in the United States, Great Britain, Denmark, Sweden and more recently Australia.

We have encountered all the difficulties in production, especially in filtration described by Dr. Bodian. In safety testing, too, we have met with them. Dr. Malherbe and his team have been particularly worried by the occurrence of smearing agents in the tissue cultures. This difficulty has been solved by preparing trypsinized cells from single kidneys. In the potency tests after trying baboons and monkeys, we have preferred the guinea pig test as described by Sen Gard. We learned the hard way and it is gratifying now to report that production is going more smoothly.

So far about 500,000 children have been vaccinated in South Africa. Reactions to the vaccine have been few and none were very serious. The campaign as carried out in the face of the worst epidemic that South Africa has experi-



enced. The occurrence of coincidental cases and they are bound to occur has given rise to much anxiety on occasion. The protection conferred by the vaccine seems to be similar to that achieved in the United States where as we have heard from Dr. Langmuir about 75 per cent of those children who otherwise would have contracted paralytic poliomyelitis do not. This is a notable achievement. But what of the remaining 25 per cent who are not protected? Can we do anything to protect them? That is an important outstanding question.

Then how should we deal with an impending epidemic? From Dr. Langmuir's paper we learn that vaccination with the killed vaccine in the face of such an epidemic did not bring it to an end. That was our experience also. Could it be brought to an end in any other way? This is another important question to which an answer is urgently needed because in the immediate future many countries will be faced with impending epidemics.

And finally it is clear from our findings and also from the more extensive studies in the United States that vaccination with inactivated vaccine does not prevent infection. This may be a happy circumstance because it does not deny the children who are vaccinated the protection which results from natural infection.

PROF. DR. HAAS. Dr. Bodian has stressed the importance of filtration for the production of vaccines particularly those which are free of viruses which are likely to multiply. So far in Germany we have used poliomyelitis virus grown in trypsinized cultures which had been obtained from rhesus monkeys. As far as the filtration is concerned from the beginning we used a certain number of Seitz filters or a certain number of combinations of Seitz filters and membrane filters. Quite apart from the asbestos contents of the levels there is nevertheless a certain amount of loss of the virus. These however may be at least reduced as far as that loss is concerned by using a proper gradation of filters so that this does not really play an important part and we were able to use a certain combination of filters so that we really did not have any great losses.

According to our experience we feel that you can also obtain better results by centrifugation and this may bring about in certain cases an in-

terruption of filter losses. During the inactivation larger losses may occur, in fact larger than before or after activation. Dr. Gard in his last figure has already referred to our experience and has shown that our values are quite close to his own findings. Nevertheless I should like to point out that the main difficulty resides in the fact that in connection with the safety of vaccines we have to say something about concentration whereas we do not really measure the concentration exactly and accurately. Consequently the important point of view is probably an approximation rather than an accurate figure. Dr. Gard referred to the immune biologic processes and he spoke about other effects that may take place. The aluminum hydroxides have been used by us or at least we have examined this technique and found that by adding aluminum hydroxide in small quantities we were able to obtain effects which increase immunity at least in tests with mice.

Vaccines with aluminum hydroxide added enabled mice to resist intracerebral Type 2 infection better than mice immunized with vaccine prepared without aluminum hydroxide. We were able to demonstrate in the rabbit that the addition of aluminum hydroxide may bring about a slowing down of the neutralizing effect. However the concentration came to higher titers and after several months of observation we found that it was always higher than the concentration of antibodies which had been obtained from animals and had been immunized without the addition of aluminum hydroxide.

PROF. DR. HALLAUER. The prevention of paralytic poliomyelitis through uncontrolled mass vaccination is a problem which in the opinion of many public health authorities has been solved for all practical purposes excepting possible technical improvement. Therefore discussion concerning the mode of action and critique of the efficacy of the vaccine is considered in certain quarters as obsolete. However since Salk himself in earlier publications and again in his paper today has examined the theoretical basis of polio vaccination and as a result has come to the optimistic conclusion that his procedure not only duplicates but surpasses the protection effects of naturally acquired immunity it seems permissible for a neutral observer to comment on this question.

1 It is obvious that no fundamental differences exist between Salk vaccination and other vaccination procedures which employ nonliving antigenic material with respect to preparation of vaccine method of immunization and development of antibodies. As a matter of fact no radically new observations were discovered in the perfection of Salk's vaccine and its mode of action follows without mystification well-established principles. The only surprise perhaps was the discovery that inactivation of polio virus through formalin apparently does not take place as a first order chemical reaction which in turn makes it impossible to eliminate the very last active virus particles from the preparation.

2 Because of these facts there is no reason to assume that the Salk vaccination should not be subject to the same inherent limitations of efficacy which characterize all similar vaccination procedures with the added importance that failures in this field are even less acceptable because of the slight risk of exposure. As is well known the protective effect stands in some correlation with the antibody level in the blood stream; the persistence of which can be guaranteed only by periodic revaccination or subsequent subclinical infection.

3 To what extent such humoral immunity is actually protective does not depend on the immunization procedure but is governed by the particular properties of the infectious agent: its tissue tropism, toxin formation, etc. The assumption has been made that the antibody barrier in the circulation is sufficient to intercept polio virus on its way to the CNS because of the further assumption that most strains utilize the hematogenic pathway of infection exclusively. Whether this apodictic pronouncement is correct must appear doubtful if for no other reason than because of the fact that Bodian himself has documented with equal persuasiveness both neurogenic and viremic channels of virus transfer in his publications of 1940, 1941 and 1952, 1955. It may be added that maximal circulating antibody levels do not have an immediate sterilizing effect but may permit the persistence of active virus in the blood and the internal organs for at least 8 to 10 days, a fact well established for fowl plague, yellow fever, foot and mouth disease virus and others.

4 A comparison of naturally acquired immunity with the Salk vaccination shows funda-

mental differences with respect to the mechanism of immunity and the degree of protection. The superiority of living over nonliving antigens depends less on possible advantages of quantity or quality than on the pattern of distribution in accordance with specific organ or tissue affinities. In the process of infection it is those tissues that function as portal of entry, avenues of dissemination and finally as a target of the lesion which are actively immunized with the production of histogenic immunity. Under these conditions the liberation of humoral antibodies appears as a secondary diagnostic reaction and the immunologic barrier lies in these same tissues which are directly involved by the infectious process and not in the blood. Natural immunization against poliomyelitis probably is protective as a rule because of the development of a tissue immunity of the lymphatic apparatus of the gastro-intestinal portal of entry. The possibilities of effective local immunization with killed antigen against enteric organisms were demonstrated successfully some 50 years ago by Metschnikoff and Besredka. In view of these facts it seems not unlikely that enteric application of formalinized polio vaccine may succeed better than intramuscular injection of the antigen. Such procedure might give a more durable local immunity and the problem of the mode of transfer of the virus to the CNS would become essentially irrelevant with this form of vaccination.

DR MURRAY: The data presented by the first three papers here today have shown that the field experience with poliomyelitis vaccine indicates that poliomyelitis vaccine as now used continues to be a highly effective agent in protecting against paralytic disease.

However, scattered fragmentary observations have suggested that commercial vaccines have not been eliciting antibodies in an appreciable proportion of triple negative children who have received 2 injections.

With such observations in mind a controlled study of a number of commercially produced vaccines was undertaken in order to determine the antibody response in triple negative children. The experimental design called for the inoculation of infants—these were 6 months to 3 years of age—in 4 widely separated cities. Triple negative subjects of older age groups were

not available. Two injections were given 1 month apart and a bleeding was taken 1 month after the second injection. It is planned to take a further bleeding after a third injection. Assays were done in the laboratory animals using both monkeys and chicks which we are using now for comparison.

So far complete results are available only through the second injection in the case of only 4 vaccines of the 9 under study. The scatter of antibody responses is shown in Figure 63.

An attempt was made to compare these responses with those which were obtained in the 1954 field trial where of course 3 injections were used. It will be remembered that the correlations obtained in the field trial between protection against disease and antibody responses enabled a classification of the vaccines used at that time into several categories from good to poor. The results in the present study were compared with those in the published reports of the field trial in terms of percentage conversions at 1/16 or above. The figure 1/16 was chosen because the groupings were different in the two studies. These results are shown in Figure 64. Two additional vaccines E and F on which partial results are available are included as a matter of interest. These are the shaded bars. Vaccine A and A' were identical except that Vaccine A was used in one area and Vaccine A' in four. It will be seen that in the case of the

vaccines for which we have complete information that responses to Type 2 were in general equal to or better than those encountered in the field trials. The Type 1 responses were generally equivalent to those classified as fair to moderate in the field trials while the Type 3 antibody responses of these vaccines compared rather unfavorably with the vaccines used in the field trial falling into the poor to fair categories.

In general the animal tests correspond to the results shown in children and the figures obtained in monkeys correlate very well with those obtained in chickens.

The present data if taken out of context with the other information reported today might be considered discouraging. However all things considered the situation appears to be the following.

Two injections of ordinary commercial vaccine leave a high proportion of triple negative children without detectable Type 1 and Type 3 antibodies. Nevertheless protection against paralytic disease is induced by this procedure. And it is worth noting that preliminary observations of others indicate that completion of the course of immunization by a third injection of commercial vaccine induces antibodies in all 3 types in the majority of children. However the present results do re-emphasize the desirability for increasing the immunizing capacity of the Type 1 and the Type 3 components of the vaccine.

I would remark about Dr. Bradford Hill's

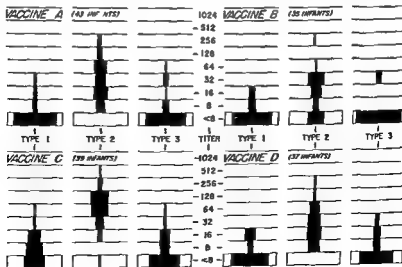


FIG. 63. Polio vaccine lot potency investigation.

comments relative to 2 versus 3 injections—we would all feel happier if we could show that they actually had antibodies after 3

DR. PENTTINEN: I will show one chart (Table 29) showing the potency of 2 lots of polio vaccine used in Finland as measured by antibody production in vaccinated children. As you will see from the table, the 1954 experimental vac-

cine used in Finland had a poor antigenicity. As regards Type 1, you see that only 12 per cent developed antibodies. They were children who did not have any antibodies before the vaccination and also the control group indicated that the increase was not due to vaccination but to the polio epidemic. It was completely ineffective against Type 1. Type 2 results and Type 3 results are better.

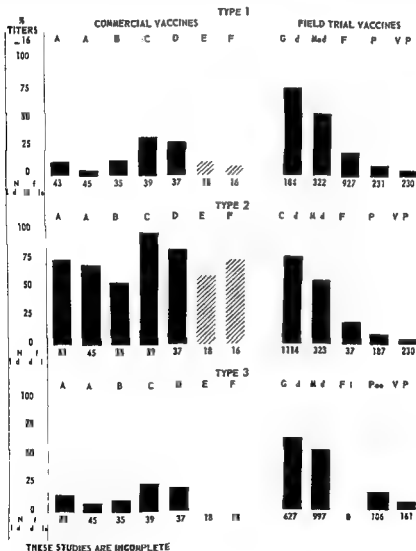


FIG. 64. Comparison of human antibody responses for several current commercial poliomyelitis vaccines and field trial vaccines of varying effectiveness.

TABLE 29 RESULTS WITH 1954 AND 1957 VACCINE SECOND BLOOD SPECIMENS COLLECTED 2 WEEKS AFTER LAST INJECTION ONLY NEGATIVES IN THE FIRST TEST PRESENTED

VACCINE	TYPE 1			TYPE 2			TYPE 3		
	NUMBER TESTED	+	PER CENT	NUMBER TESTED	+	PER CENT	NUMBER TESTED	+	PER CENT
1954	134	16	12	92	31	34	63	16	25
1957	46	25	54	48	37	77	48	25	52

Control group indicates that the increase was due to polio epidemic

In 1957 the vaccine is much better. The antibody response against Type 1 is 54 per cent, Type 2 77 per cent, and Type 3 52 per cent. They all are children who did not have any antibodies before the vaccination.

It is possible that the 1954 experimental vaccine was damaged by merthiolate or due to shipment. In 1957 commercial vaccine as you see was much better although as regards measurable antibody formation not complete when children with no antibodies before vaccination were in question.

PROF. DR. PETTE: On the basis of experimental studies conducted at the Institute of the Foundation for Research in Spinal Paralytic Poliomyelitis and Multiple Sclerosis, I would like to make the following comments:

1. In his paper Dr. Salk concerned himself with the question of Type 1 hyperreactivity in Type 2 immunity. Figure 65 shows the results of personal investigations. Of 150 patients who had developed paralytic or aparalytic meningitic poliomyelitis following an infection with Type 1

polio virus, 41 exhibited antibodies against all 3 types, 35 exhibited antibodies against 2 types, and 74 exhibited antibodies against 1 type. Type 1: Of the 35 patients with antibodies against 2 types, 11 had antibodies against Types 1 and 2, and 24 had antibodies against Types 1 and 3. Altogether, therefore, 52 of 150 patients had before infection with Type 1 antibodies against Type 2, 98 not exhibiting such Type 2 antibodies. If the paralytic and nonparalytic cases are considered separately, we find that this ratio remains the same. From this we conclude that the immunobiologic hyperreactivity to Type 1 that Dr. Salk assumes to be present in Type 2 immunity and the protection resulting therefrom should not be rated too highly.

2. Immunization experiments with viruses of the EMC group in rabbits and guinea pigs have shown that here, too, the antibody content of the serum is limited in its dependence on the introduction of active antigen. No specific antibody maximum can be increased by further administration of antigens.

3. We have investigated experimentally the question of whether the increase in antibody titer after polio vaccination could be a nonspecific anamnestic reaction. Following encephalography with air, smallpox vaccination, diphtheria toxin, and tetanus toxin inoculation in 30 subjects, no increase in polio antibodies was noted.

4. We agree with Dr. Bodian that tissue cultures to determine the presence of any virus in the vaccine can be more useful than the monkey test, but share his view that we cannot dispense with the latter, particularly in view of the desirable maintenance of continuous safety controls. Evaluation of neurohistologic changes requires, however, especially extensive experience in neuropathology and the most critical judgment.



FIG. 65. Antibodies against poliomyelitis after illness with Type 1 (150 patients—paralytic, aparalytic meningitis).



FIG 66 Encephalomyelitis in a monkey

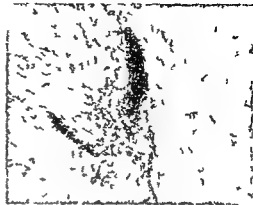


FIG 67 Encephalomyelitis in a monkey

5 With regard to Dr Langmuir's paper even if it is true that neuro allergic reactions are relatively rare following polio vaccination since severe forms of postvaccination encephalomyelitis seem to have been observed in only isolated cases more attention than heretofore should be given to the *formes frustes* of allergic encephalomyelitis i.e. those cases in which collapse occurs immediately after the second or third injection. It should not be forgotten that in cases of collapse lasting for several hours cerebral circulatory disturbances in the gray substance of the central nervous system particularly in the cerebral cortex can develop. From experience with other types of disturbances that occur following infection and vaccination anaphylactically or with disturbances of the delayed type we know that such children can later undergo psychic changes or also react with epileptic attacks. It is the clinician's duty to draw attention to this possibility. Finally it is generally accepted biological knowledge that repeated injections of foreign protein into an organism can lead to allergic reactions not only in the central nervous system but in other organs as well.

Figures 66 and 67 will serve to demonstrate an instructive experimental finding yet one which of course should not be generalized. Of 6 monkeys inoculated with Salk vaccine 1 of the animals became sick a few days after the third injection exhibiting myelitis. Histologic investigation revealed the classical picture of a perivenous encephalomyelitis in addition to a nodular pulmonary tuberculosis which pathogenetically speaking must have been a decisive factor in

the development of the encephalomyelitis. An interesting fact is that the antibody titer of the sick monkey was considerably higher than that of the 5 other animals vaccinated. This basically important experimental finding in the animal indicates that one should proceed with reserve in considering polio vaccination for children suffering from a chronic infectious disease especially tuberculosis. The same is true of children who have a marked tendency to severe allergic reactions. In this connection the latest finding reported by Uehlinger of Zurich (*Schweiz. med. Wchnschr.* p 813 1957)—that of a case of anaphylactic reaction following poliomyelitis vaccination in a 4½ year-old child who developed a condition resembling Landry's paralysis 3 days after the second injection and died—certainly merits particular attention.

In closing I mention the results of antibody determinations in a number of children who had not shown any antibodies against one of the 3 polio virus types (so-called *miss*) prior to inoculation. Two weeks after the second administration of the vaccine the antibody titer was found to be very good against Type 2, good against Type 1 and fair against Type 3.

PROF D. STUART HARRIS My chief comment as one who has been concerned in the question of field trials relates to the great debt which we all owe to those who planned and executed the Francis field trial in the United States. We acknowledge this because the scientific impeccability of this trial enabled others of us faced by the problem of the introduction of the use of the

Salk vaccine to concentrate on certain questions which appeared to require additional answers. I hope that our experience in Great Britain in relation to the protection in the under school age child and also with the Brunenders virus as an alternative to the Mahoney strain will be useful.

To turn to the problems of the future, pride of place in my view should undoubtedly go to the question of how does the vaccine work? The remarkably small amount of virus content in the vaccine contrasts with its efficiency as a method of inducing antibodies. Also as already mentioned this afternoon after use of the vaccine one can still obtain infection of the alimentary tract even though paralysis does not occur. And this fact contrasts with the effect of natural infection. It is remarkable perhaps that nonparalytic disease is not apparently affected by the vaccine though the ECHO and the Coxsackie viruses undoubtedly interfere with the assessment of the vaccine against this type of disease. In the British trial there was some reduction of nonparalytic disease in cases from which actual poliomyelitis viruses were obtained. The evidence was just suggested. One can only speculate concerning this question of whether the vaccine induced antibody prevents blood stream invasion or that it operates at a neuronal level either at the periphery or within the areas of the blood brain barrier. These are questions which the immunologist must solve no doubt in the future and also additional questions concerning the duration of immunity and the hyperreactive state.

Unquestionably one of our future problems is going to be: How can a population be induced to accept vaccine to the required level for the complete control of the disease? Nowadays one third of all of our cases of poliomyelitis in Britain occur in those over 15 years of age. How can we persuade adults to be immunized to the fullest possible extent during the early phase of a mass campaign? This is a matter which undoubtedly will be solved in different countries by different ways but in a country such as Great Britain where mass anything is questioned it will require a considerable effort to persuade adults to be immunized. Perhaps it may yet be found that the disease incidence will wane in other groups once the most susceptible infants and children have been immunized.

The third question for the future seems to be

What should be the earliest age at which vaccine should be administered? In Britain the methods and the timing of inoculations with antigens such as diphtheria and whooping cough are being revised in order to aim at the prevention of provocation poliomyelitis. It is clearly advantageous to give the vaccine at the earliest possible age compatible with an adequate antibody response. Is 6 months too early because maternal antibodies may still be present or would it be safer to give the vaccine later? In view of Dr Langmuir's figures of the continued high attack rate of poliomyelitis in infants under 1 year it seems essential to give the vaccine at as early an age as possible. This question probably requires laboratory study.

Finally several people have mentioned the problem of the relative power of protection of 2 doses as compared with 3 doses of vaccine. Whatever may be said about the antibody response this is really a matter which requires further field study in order that complete verification can be obtained.

Dr TOBIN: Dr Bradford Hill has said that the Mahoney strain has been replaced by the Brunenders attenuated strain in the production of British vaccine. The Type 2 and Type 3 strains have remained the same as those used by the Americans. He has also said that the British vaccine has given good protection. It is not surprising then that it is also antigenic.

Figure 68 shows the geometric mean titers obtained with the 3 batches of British vaccine produced by Dr Wood and his colleagues at Glaxo and which were used in the British field trial of last year. They are compared with a good American commercial vaccine kindly provided by Eli Lilly and Company. In the top line of pillars is the Type 1 response. The antigenic response produced by the Brunenders component compares well with that produced by the Mahoney one contained in the original vaccine. The Type 2 and 3 components being produced by the same strains as the American vaccine have also given comparable results. These are the results in monkeys.

We have also obtained results with the British vaccine in triple negative children. These children I think are definitely triple negative because the methods used for estimating the antibodies are very sensitive. Although we have been using roller tube technique we have kept the

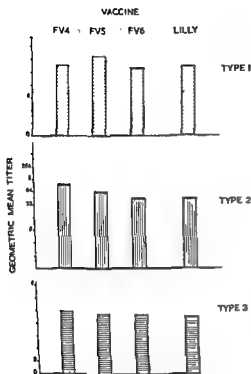


FIG 68 Geometric mean titers obtained with 3 batches of British vaccine and 1 American vaccine

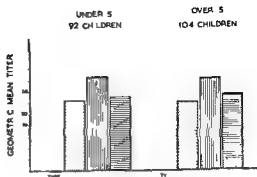


FIG 69 Geometric mean titers obtained in triple negative children

virus and the serum dilutions in contact for 3½ hours at 30 C as this seems to increase the sensitivity of the test

The children who were triple negative were between the ages of 1 and 9 and we have divided them into those over 5 and those under 5 as the antigenicity in these 2 groups was of interest (Fig 69). The Type 1, 2 and 3 components have all given a good response in both age groups. In spite of the championship of the Mahoney strain by Dr Bodian and Dr Smadel the Brunenders strain is not so bad for producing killed polio vaccine. However the introduction of an attenuated strain has made us alter our safety testing procedures which are almost the same as the American procedures in one respect. This strain if inoculated into cynomolgus monkeys in doses of 100 000 to 1 000 000 tissue-culture doses will produce viremia in nearly all monkeys but no invasion of the central nervous system occurs. As you can see it is attenuated.

However with the simultaneous injection of cortisone 10 to 20 tissue-culture doses will regularly produce infection in cynomolgus monkeys with degree of involvement of the central nervous system depending on the dose of cortisone. For this reason we are retaining the monkey test in the safety test of our vaccine also keeping in mind of course that we have to test for Types 2 and 3. But the sensitivity of these monkeys under the treatment of cortisone in direct titrations is much of the same order as one gets with Mahoney strains in normal monkeys.

However with this Brunenders strain there is some inhibition when a vaccine is inoculated simultaneously and we are not quite yet certain of the dose to be used in order to get the required sensitivity as compared with the amount of vaccine tested. If any reversion to more virulence does occur and live virus may have been missed in a monkey the monkey test itself will automatically be one more sensitive and will act as a second safeguard in case for some unknown reason the tissue-culture tests do break down.

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# Vaccination Against Poliomyelitis

TUESDAY MORNING, JULY 9, 1957

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South African Institute  
for Medical Research  
Johannesburg

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# Vaccination with Modified Active Viruses

DR HILARY KOPROWSKI

The careful and patient evaluation of immunization with attenuated living polio viruses during the past 7 years now has reached a point where large mass clinical trials have to be taken into serious consideration. If this method of immunization which may lead to the eradication of poliomyelitis virus as a constant menace to the world community is effective then there is all the more reason to be fully cognizant of all the factors involved in studying the problem.

The purpose of this presentation is to examine critically the work done with the attenuated polio viruses now available which judging from all experimental data are probably as good as they ever will be and hence should be given a fair trial.

Type 1 SM strain was originally attenuated by intraspinal passages in mice. Only 3 human subjects received the material representing the 28th mouse brain passage of the virus (Table 30). This strain has been passed through 14 serial chick embryo tissue-culture transfers but because the 14th passage failed to show cytopathogenic properties for any of the tissue culture systems it was assayed on 5 alternate passages between monkey kidney tissue cultures and chick embryo tissue cultures were made in order to obtain and maintain a demonstrable concentration of the virus. This 5th alternate passage was made into a large chick embryo pool called SM N 90 strain. Its pathogenic properties for either the rhesus or the cynomolgus monkeys injected intracerebrally were found to be of very low order although occasionally lesions were found in the central nervous system of monkeys who apparently remained symptom free after the inoculations. Thus we may assume that by passage through several generations of nonprimate hosts a population has been obtained still probably nonhomogenous which was predominantly characterized by decreased neurotropism for monkeys.

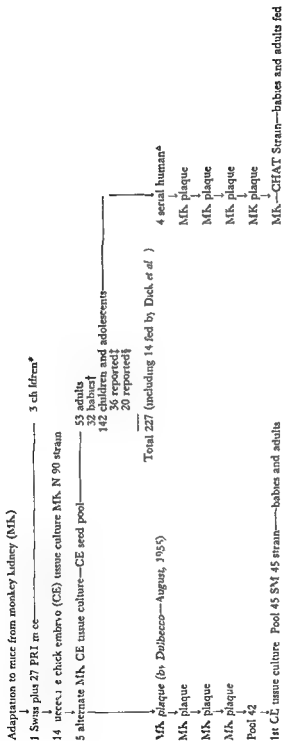
At this point it was decided to apply the technique of purification of the strain intro-

duced by Dulbecco *et al* by studying single plaque progenies. The first plaque of the material was originally isolated by Dr Dulbecco and in my laboratory this material was further passaged through 4 single plaque passages before it was made again into a pool for study. This subline named SM 45 strain was submitted to tests for neurotropism by intracerebral and intraspinal inoculation of cynomolgus monkeys and was found to be attenuated to a high degree as evidenced by very low intraspinal pathogenicity for monkeys.

The history of the CHAT strain which is summarized on the right of Table 30 is also of interest. This line originated from the 4th serial human passage of the N 90 pool. Fecal virus was plated out in monkey kidney monolayer and single plaque progenies were transferred through 3 additional passages. We have obtained an indication of a remarkable degree of attenuation. In Table 31 you will see the last 3 plaques examined and analyzed and you will notice the very low intracerebral/intraspinal pathogenicity of the plaques which are numbered 39, 41 and 42. This was substantiated further by the absence of clinical signs of illness in 5 chimpanzees injected intraspinally with this substrain and by the lack or minimal presence of histopathologic lesions in the anterior cords of monkeys injected with this strain.

Results of titration of the CHAT strain in monkeys inoculated with serial dilutions are shown in Table 32. The lack of pathogenicity after intracerebral inoculation is confirmed by the absence of histologic lesions in the central nervous system. The histologic examination of spinal cords of monkeys injected with the large  $10^{6.5}$  tissue-culture doses revealed an interesting phenomenon namely that the paralysis observed in 1 of the monkeys was caused probably by trauma since no neuronal lesions characteristic for poliomyelitis were observed. In 2 other monkeys of which only 1 showed signs of paralysis after injection with  $10^5$  tissue-culture

TABLE 30 HISTORY OF THE ATTENUATION OF SM VIRUS (TYPE 1)



Mk = M L J Lney

CE = Cl L mbryo

L p k rt J P oc Soc Exper B 1 & Med 86 244 1954

† L p w k J JAMA 162 1281 1956

‡ L p w k J JAMA 160 154 1956

§ L p w k J JAMA 160 154 1956

¶ L p J Brit Med J N 5010 p 65 195

N 1 n T L *et al.* To be published

TABLE 31 SM CHAT PLAQUE LINE

Final Stool Conc (5.7)*			
Plq 1 IC 1/4† [1 Min] ‡ IS 1/4 [1]	Plq 8 IC 0/4 [0] IS 0/4 [1]	Plq 9 (5.2) IC 0/4 [0] IS 0/4 [0]	Plq 10 IC 0/4 [0/3] IS 0/4 [0/3]
Plq 13 (7.2) IS 2/5 [1]	Plq 14 IS 2/5 [0]	Plq 15 IS 0/5 [0]	Plq 16 IS 0/5 [1]
Plq 18 (7.2) IS 1/4 [1]	Plq 19 (7.7) IS 0/4 [1]	Plq 20 (8.2) IS 0/4 [0] IS 0/5 (Chumps)	Plq 21 (7.0) IS 1/4 [1 Min]
	Plq 23 IS 2/4 [0]	Plq 24 IS 0/4 [1 Min]	Plq 25 IS 2/4 [0]
			Plq 26 IS 2/4 [3]
			Plq 27 IS 1/5
Plq 34 (8.2) IS 1/4 [0]	Plq 35 (7.5) IS 1/4 [1]	Plq 36 (7.5) IS 1/4 [0]	Plq 37 (7.0) IS 0/4 [1]
	Plq 39 (7.2) IC 0/5 [0] IS 0/5 [0]	Plq 41 (6.5) IC 0/4 [0] IS 0/5 [0]	Plq 42 (6.7) IC 0/5 [0] IS 0/4 [0]
			Plq 38 (6.7) IS 1/4 [1]

TABLE 32 PATHOGENICITY FOR MONKEYS OF THE CHAT STRAIN OF TYPE 1 VIRUS

ROUTE	LOG OF TCD, IAC	PARALYTIC RATIO	RATIO OF CNS LESIONS	CELLULAR LOSS PER CENT	
				CERV	LUMBAR
Intracerebral	7.2				
	6.2	0/4	0/4		
	5.2	0/4	0/4		
	4.2	0/4	0/4		
Intraspinal	6.5	1/4	0/3*		
	5.5	1/4	2/4†	12	20
	4.5	0/4	0/4	10	16
	3.5	0/4	0/4		

\* Heflig v m n t n f l symptomatic monkey was not fed  
 † T m u n a c l e s n b e r e d n b t h n n

doses of virus histologic examinations showed presence in minor degree of destruction of anterior horn cells at lumbar and cervical enlargements of the spinal cord. The remaining results were negative.

Investigations of properties of an attenuated strain of poliomyelitis virus to be comprehensive obviously should include examination of the virus after passage through the human intestinal tract. In the course of years of observations it became apparent that the virus population multiplying in the alimentary tract of an infant may undergo some increase in neurotropic properties but that by passage through

the intestinal tract of an adult it does not. Consequently monkeys were injected with fecal extracts originally obtained from infants fed this CHAT virus. The results shown in Table 33 indicate that in the first case a pool made of fecal virus obtained at different time periods after feeding failed to cause paralysis or spinal cord lesions in monkeys injected either intracerebrally or intraspinaly. Total fecal output of the second case marked TA obtained during the entire period of alimentary infection was processed and the concentrate titrated in monkeys. No paralysis was observed in monkeys injected intracerebrally and only slight paralysis

TABLE 33 FATE OF MONKEYS INJECTED WITH CHAT STRAIN OF ATTENUATED TYPE 1 VIRUS

SUBJECT	MATERIAL	DAYS AFTER INJECTION OF VIRUS		INTRACEREBRAL			INTRASPINAL		
		3-6	10-13	TCD INOC	PARALYTIC RATIO	LESIONS	TCD INOC	PARALYTIC RATIO	LESIONS
BO	Feces	17	20 24 27	3.4	0/5	0/5	2.7	0/5	0/5
TA†	Feces	1	26†	3.7 2.7 1.7 0.7	0/4 0/4 0/4 0/4	NT	3.2 2.2 1.2 0/2	2/4 1/4 0/4 0/4	NT
GA‡	5th TC	1	36†	7.4	0/5		6.7	0/5	

R by 87 d; old at time of subinjection  
 † B by 28 d; old at time of subinjection  
 ‡ T at fecal virus output and fecal culture  
 § B by 80 d; old at time of subinjection

in one of the lines was seen in each of the 3 monkeys tested immediately. To overcome criticism that concentration of the fecal virus was too low to reveal changes in the character of the virus, total fecal virus obtained from a third monkey, named G.A. on the table, was passed through 5 monkey kidney tissue-culture passages in order to increase the titer of the virus. The results indicate that even large doses of the CHAT strain, after passage through human intestinal tract, failed to cause paralysis in monkeys.

Study of characteristics of another progeny of the SM Type 1 virus, the 45 strain, which was derived also through single-plaque passages (Table 30) is now demonstrated in Table 34. In addition to the inoculum also the total fecal virus obtained during the period of alimentary infection from a 32-day-old baby was tested in monkeys. In order to raise the concentration of the virus, 4 additional tissue-culture passages were made and then used as inoculum. The results failed to show any increase in pathogenicity for monkeys inoculated intracerebrally. Two monkeys showed paralysis after intraspinal inoculation which does not necessarily indicate increased neurotropism, since at present there is no strain of attenuated polio virus available which is completely devoid of paralytogenic properties after intraspinal inoculation of monkeys.

The attenuated Type 3 polio virus also has been subjected to rigid tests after passage through the alimentary tract of infants. Originally it was

derived from a stool of an asymptomatic child and purified through 6 single-plaque passages, as shown in Table 35. The brackets in this table refer to the histologic examination and the lesions found in the monkey's central nervous system. Fate of the virus following its administration to a 72-day-old infant is shown in Table 36. The original feces contained too little virus for the negative results to be of significance. However, the 2 tissue-culture passages of fecal virus failed to show any increase in the paralytogenic properties of the virus even though large concentrations of infectious material were injected into monkeys either by the intracerebral or intraspinal routes.

The currently employed Type 2 polio virus has been derived from an infant who ingested one of the MEF<sub>1</sub> substrain viruses. Because of lack of cytopathogenic properties of the original egg-passaged strain, it was impossible to subject it to purification through isolation of progenies of single plaques. However, a passage through the intestinal tract of an infant yielded a virus which was "weakly" cytopathogenic but became more so in the course of repeated passages through tissue-culture medium. Following 5 passages it was possible to plate out the virus and to isolate 6 single plaques on monkey kidney monolayers. Progenies of these plaques were further purified through 5 passages and the resulting material tested for its ability to grow in developing chick embryo. Of 5 plaques 3 yielded progenies which multiplied without any difficulty in the egg. One failed to do so and the

TABLE 34 FATE OF MONKEYS INJECTED WITH SM-45 STRAIN OF ATTENUATED TYPE 1 VIRUS

MATERIAL	DAYS AFTER INGESTION	INTRACEREBRAL		INTRASPINAL	
		TCD <sub>50</sub> INOC.	RATIO	TCD <sub>50</sub> INOC.	LESIONS
Inoculum		5.5 5.2	0/5 0/5	4.5	0/5
Feces*	1-27†	9	0/4 0/4 0/4	3.2 2.2 1.2	3
4th T.C.	1-27†		0/5	7.2	

Baby 39 days old at the time

on.

† Total fecal virus output





TABLE 36 FATE OF MONKEYS INJECTED WITH ATTENUATED FOY 3 STRAIN FOLLOWING PASSAGE THROUGH HUMAN INTESTINAL TRACT\*

MATERIAL	DAYS AFTER INGESTION	INTRACEREBRAL			INTRASPINAL		
		TCD <sub>50</sub> INOC	PARALYTIC RATIO	LESIONS	TCD <sub>50</sub> INOC	PARALYTIC RATIO	LESIONS
Feces	3-9	2.4	0/4	0/4	1.7	0/4	1/4 <sup>†</sup>
5th T.C.	3-17 31-48	7.2	0/4		6.5	0/4	
		6.2	0/4		5.5	0/4	
					4.5	0/4	
					3.5	0/4	
					2.5	0/4	
	3-52†	7.2	0/4		6.5	0/4	

Baby 78 days of age at the time of virus administration

† Total fecal virus output concentrated and used as seed for tissue culture passages

TABLE 37 COMPARATIVE PATHOGENICITY FOR CYNOMOLGUS MONKEYS OF 2 ATTENUATED STRAINS OF TYPE 1 POLIO VIRUS

ROUTE	STRAIN	LOG OF TCD <sub>50</sub> INOC	NUMBER OF MONKEYS		
			INOCULATED	PARALYZED	WITH SPECIFIC CNS LESIONS
Intracerebral	CHAT	7.2	4	0	0
		6.2	4	0	0
		5.2	4	0	0
		4.2	4	0	0
	N 90	5.0	4	0	1
		4.0	4	0	0
		3.0	4	1	3*
		2.0	4	0	2
		1.0	4	0	0
	Intraspinal	CHAT	6.5	4	1
5.5			4	1	2*
4.5			4	0	0
3.5			4	0	0
N 90			3		2
			3		1
			3		1†
			3		0

Minimal loss of anterior horn cells in the cerebellum

† Another monkey showed traumatic type of lesions

results with 1 strain were equivocal. The problems concerning this mixed virus population in relation to its characteristics in egg growths as well as other characteristics of the virus after passage through the infant alimentary tracts are being currently investigated.

The word attenuation implies that an agent has undergone changes in its characteristics for instance loss of pathogenic properties for the original host. However if an attenuated virus is to be used as a vaccine it must retain the immunogenic power of the original strain. This has not always been the case. Indiscriminate passages of the 17D strain of yellow fever virus through numerous tissue-culture generations led to the development of a strain which although infectious for mice had lost its immunogenic power for man. Are we facing a similar problem with the attenuation of polio viruses? The answer may well be in the affirmative.

Table 37 summarizes results of comparative titrations in monkeys of 2 attenuated sublines of the same Type 1 virus: the SM strain. Analysis of the results indicates that the N 90 is more pathogenic for monkeys than the CHAT strain. Paralysis occurred in 1 monkey after intracerebral inoculation and specific CNS lesions were observed in monkeys inoculated both intraspinally and intracerebrally with low concentrations of the N 90 virus in contrast with the results obtained with the CHAT strain of lesser neurotropism in monkeys.

The results of ingestion of these 2 strains of virus by a group of infants is shown in Table 38. These were groups of infants who had congenitally acquired passive antibodies and the concentration of antibodies was similar in the two groups who were approximately the same age. The 2 strains were administered orally: the less attenuated N 90 strain and the more attenuated CHAT strain. The less attenuated N 90 subline proved to be an excellent antigen. Seven infants ingested  $10^4$  to  $10^5$  tissue-culture doses of virus and of those 7 only 3 failed to develop active immunity. Higher concentrations of this virus immunized every subject. In marked contrast the seemingly more attenuated CHAT strain failed to immunize 5 out of 5 infants who received  $10^{1.6}$  to  $10^{1.2}$  tissue-culture doses of the virus and 1 out of 3 infants failed to develop active antibodies following ingestion of  $10^{4.5}$  to  $10^5$  tissue-culture doses. Only when given in

TABLE 38 COMPARATIVE ANTIGENICITY OF 2 STRAINS OF TYPE 1 POLIO VIRUS AT DIFFERENT STAGES OF ATTENUATION ADMINISTRATION TO INFANTS LESS THAN 6 MONTHS OLD

AMOUNT OF VIRUS FED IN TCD <sub>50</sub> (RANGE)	RATIO OF INFANTS FAILING TO REACT AFTER FEEDING VIRUS STRAIN	
	N 90	CHAT
2.5-3.5	3/7	NT
3.6-4.5	0/7	5/5
4.5-5.5	0/9	1/3
> 5.5	NT	0/5

Expressed in terms of 10<sup>1.0</sup>  
 † Number of infants failing to respond  
 denominator = number of infants receiving virus  
 ‡ All 3 infants ingested 10 TCD<sub>50</sub> serially administered in 10 TCD<sub>50</sub> resulted in no neutralizing response

excess of  $10^5$  tissue-culture doses did the CHAT strain seem to elicit uniform antibody response.

There were 2 logs difference in immunogenic capacity between the more attenuated strain and the less attenuated strain of the same subtype. You need  $10^4$  to  $10^5$  tissue-culture doses to cause immunity after the original strain and you need  $10^6$  tissue-culture dose of the more attenuated to provoke immunogenic response.

Granted that this series is small and the results may be characteristic only for this particular series nonetheless it is conceivable that it may represent a general trend in the field of attenuation of polio viruses. This ultimately may put the advocates of superattenuation in an untenable position of recommending administration of gallons of polio virus to immunize 1 child and the creation of new criteria of attenuation may also have to become less exuberant in light of experimental facts. In this frantic search for apathogenicity one should not forget that a balance between modification of characteristics and retention of antigenic properties has to be maintained in order to bring the work to its logical fruition.

It is now more than 7 years since the TN strain was administered for the first time to a human subject with development of homotypic antibodies following its ingestion. Data summarized in Table 39 show persistence of antibodies in sera of 9 children who were fed the virus 7 years ago. In 5 subjects there was no difference in antibody levels between specimens

TABLE 39 PERSISTENCE OF ANTIBODIES AGAINST  
TYPE 2 POLIO VIRUS IN SERA OF INDIVIDUALS  
FED TN STRAIN

IND. VIDUAL NUMBER	MINIMUM PROTECTIVE TITER* OF SERUM OBTAINED MONTHS AFTER ADMINISTRATION OF VIRUS	
	1-11 Mos	60-72 Mos
2	20 (1)†	20 (72)
3	16 (2)	20 (72)
9	22 (3)	20 (72)
5	19 (1)	12 (72)
7	24 (1)	24 (72)
8	24 (1)	25 (72)
13	16 (1)	12 (60)
14	18 (13)	12 (72)
10	24 (1)	18 (60)

Log<sub>2</sub> to base 10

† Number in ( ) = month after feeding

collected 1 to 3 months after virus ingestion and 6 years later. In 4 cases a slight decrease was observed which is probably insignificant insofar as insusceptibility to alimentary infection is concerned.

The homotypic antibody levels observed after

ingestion of attenuated Type 1 virus shown in Table 40 have been maintained with minor fluctuations throughout almost a 3 year observation period.

TABLE 40 PERSISTENCE OF ANTIBODIES AGAINST  
TYPE 1 POLIO VIRUS IN SERA OF INDIVIDUALS  
FED SM STRAIN

INDIVIDUAL NUMBER	MINIMUM PROTECTIVE TITER* OF SERUM OBTAINED MONTHS AFTER ADMINISTRATION OF VIRUS		
	1 Mo	2-14 Mos	15-34 Mos
GE	24 (1)†	29 (9)	30 (28)
GO	15 (1)		18 (77)
W	21 (1)	28 (9)	25 (78)
BR	15 (1)	18 (12)	22 (26)
T	18 (1)	12 (12)	12 (31)
M	22 (1)	12 (14)	24 (15)
BO	25 (1)	18 (12)	18 (31)
SC	10 (1)	25 (2)	24 (33)
JC	15 (1)		25 (34)

Log to base 10

† Number in ( ) = month after feeding

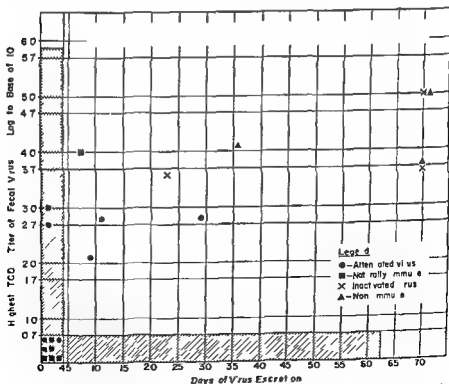


FIG 70 Duration of alimentary infection and concentration of fecal virus excreted by children infected orally with  $10^6$  TCD<sub>50</sub> of Type 1 virus

TABLE 41 RESISTANCE OF INTESTINAL TRACT OF CHILDREN TO INFECTION WITH  $10^7$  TCD<sub>50</sub> OF TYPE 1 VIRUS IN NONIMMUNE NATURALLY IMMUNE AND IN CHILDREN VACCINATED WITH ATTENUATED VIRUS\* AND WITH INACTIVATED VIRUS†

PREVIOUS HISTORY	ALIMENTARY INFECTION			SERUM ANTIBODY TITER		
	SUBJECTS EXCRETING VIRUS		DURATION CARRIER STATE	HIGHEST TCD <sub>50</sub> TITER OF FECAL VIRUS	BEFORE INGESTION OF VIRUS	AFTER INGESTION OF VIRUS
	LESS THAN 4 DAYS	MORE THAN 4 DAYS	DAYS			
Attenuated Virus*	7				1 11 1 16 1 16 1 16 1 23 1 712 1 1 024	1 256 1 1 024 1 16 1 11 1 512 1 246 1 362
		3	8 11 28	10 <sup>7</sup>	1 4 1 16 1 23	1 712 1 256 1 256
Natural Immunity	3				1 64 1 256 1 16	1 64 1 178 1 16
		1	6	10	1 44	1 64
Inactivated Virus†	0					
		3	21 89 97	10	1 4 < 1 2 1 64	1 1 074 1 256 1 256
Nonvaccinated	0					
		3	36 61 98	10 <sup>10</sup>	< 1 2 < 1 2 < 1 2	1 256 1 256 1 91

\* Virus 12 years before test.

† Virus 6 months before test.

We have also subjected several infants and children who were fed the virus to a refeeding of the same virus and these studies are summarized in Table 41. Note that 7 out of 10 subjects who had had a single feeding of attenuated Type 1 virus still had intestinal tract protection  $2\frac{1}{2}$  years later against a massive dose of the same virus given orally. In the remaining 3 subjects re-exposure to the same agent resulted in sporadic excretion of the virus which was still recoverable 28 days after ingestion in 1 single case. The highest concentration of the fecal

virus never exceeded  $10^7$  tissue-culture doses. Booster effect on antibody titers was observed in 6 out of 10 subjects. Antibody content in sera of 2 other subjects whose pre-exposure titers were high appeared to show a decrease but this may well be within the limits of experimental error of the test.

Resistance of the alimentary tract to infection was observed also in 4 subjects who were found to be naturally immune before exposure to Type 1 virus. Three did not excrete the virus and 1 did during 6 days' concentration of the fecal

virus on one occasion reaching  $10^{4.0}$ . No booster effect was noted in any of the 4 subjects.

In marked contrast to these results subjects who had been vaccinated with inactivated virus or were nonimmune developed an alimentary infection which lasted from 1 to 3 months with fecal virus titers surpassing by more than 2 logs those observed in the isolated instances of alimentary infection after re-exposure in the group originally immunized with living virus. Rise in antibody was considerable in both the nonimmune group and in those vaccinated with inactivated virus (Fig. 70). Although these results were obtained with a relatively small group of subjects the evidence is quite overwhelming in favor of prevention of alimentary carrier state through ingestion of living virus rather than through injection of inactive virus. Apparently there are two mechanisms of defense against poliomyelitis—one central probably operating in the presence of circulating antibodies and the other local operating in the intestinal tract and not related to the level of circulating antibodies. Inactivated virus may evoke the former the living virus may elicit both.

In considering biologic and medical problems balance of values and sense of proportion should be carefully matched. Protection of man against a disease is obtained at a price nothing in nature is given free and all efforts should be made to reduce the cost of this payment. Yet in recent years considerable hue and cry has been raised that the attenuated living polio viruses with all their numerous advantages should fulfill criteria of perfection which have never been met before and never will be in other and similar fields. There is a desire to consider any evidence of mutation almost in the light of a crime committed by the virus against nature. However is it possible to consider that the only virus particles which will never mutate are those which do not exist?

The striving for a virus strain completely devoid of any neurotropic properties may lead to an agent which retains infectivity on experimental assay but is no longer infectious or antigenic for man. In terms of such logic the strain should not cause alimentary infection and yet be able without multiplication to immunize the subject against disease by a mechanism not apparent in any other infection. The advocates of safety do not want to pay any price for immunization yet

exactly what are the costs one might have to pay for a method of immunization which would not only protect the vaccinated subject against the disease but also may lead to elimination of poliomyelitis?

From all the data accumulated in date with recently studied attenuated strains in 2 or 3 different laboratories there is no evidence that the degree of mutation of these polio viruses in the human intestinal tract or in any other susceptible system is such that it will ever lead to production of a strain which will be highly paralytogenic for man. Experimental evidence supporting such ideas should be furnished by those who propound them not by us. But let us suppose that an attenuated polio virus spreads in a community and in course of passages changes to a more virulent virus albeit there are no experimental and clinical data supporting such an idea. In endemic areas covering most parts of the world such a virus is no greater a menace to the community than the numerous other strains which are isolated from feces of completely healthy children and more often than not are highly virulent for intraneurally injected monkeys. Of the hundreds of nonimmune individuals who received attenuated polio viruses developed in the 2 laboratories none showed signs of illness. 6 serial passages through the human intestinal tract have not led to a virus which is pathogenic for man or highly neurotropic for primates. Thus there is every indication that if an attenuated polio virus were disseminated through a community unlikely as that may be it would play a prominent role in the ultimate replacement of the more virulent wild strains. If there is concern over the personal safety of the thousandth or the millionth individual who is to swallow the attenuated virus then it could be administered in the presence of antibodies acquired congenitally or with a gamma globulin injection or following vaccination with inactivated virus.

Such procedures should satisfy even the most adamant adherents of the safety first school. Strains available today for large scale clinical trials may be as good as they probably ever will be. Those of us who have worked in this field for years have to reach a point where we refuse to search indefinitely for strains which very often will fill imaginary criteria of attenuation and while doing so subjecting hundreds and thou-

sands of monkeys and chimpanzees to inoculations observations and histologic examinations

The time has come when a careful and patient evaluation of the attenuated viruses as immunizing agents against poliomyelitis may lead those who have a sense of proportion to the conclusion that the price one has to pay today for the comfort of future generations is indeed negligible

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# Progress and Evaluation of Orally Administered Attenuated Polio Virus Vaccine

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The oral polio vaccine (OPV) is a live attenuated virus vaccine. It is administered orally and is highly effective in inducing immunity. The vaccine is made from a strain of polio virus that has been attenuated by serial passage in tissue culture. The resulting virus is much less virulent than the wild virus, but it still retains the ability to replicate in the intestinal tract and to induce a strong immune response. The oral vaccine is administered in the form of drops or capsules. It is usually given in a series of three or four doses, with a booster dose one year later. The oral vaccine is highly effective in preventing polio, and it is also effective in preventing the spread of the virus to others. The oral vaccine is a major advance in the control of polio, and it has been instrumental in the eradication of the disease in many parts of the world.

has been found to reproduce the natural infectious process in the alimentary tract, there may be reason for expecting that the resulting immunity may also be as long-lasting but only the passage of time can establish whether or not such expectation is justified

Recent studies summarized in Table 4<sup>1</sup> have also shown that a single infection by attenuated strains can reproduce the resistance of the alimentary tract to subsequent infection that is found in naturally immune individuals but not in those immunized by the intramuscular injection of killed virus vaccine. Tests on 4 different groups are shown. One hundred thousand tissue-culture doses of the indicated type of attenuated virus were fed to the number of individuals in

TABLE 4. RESISTANCE OF ALIMENTARY TRACT TO INFECTION WITH ABOUT 10 PFU OF INDICATED ATTENUATED POLIO VIRUS IN IMMUNE AND NATURALLY IMMUNE ADULTS AS WELL AS IN ADULTS WHO RECEIVED SINGLE FEEDINGS OF ATTENUATED VIRUS OR 2 DOSES OF SALK VACCINE INTRAMUSCULARLY

CATEGORY	TYPE 1 LSC	TYPE 2 P 712	TYPE 3 LEON
When tested for high-avidity antibody	17/19*	15/15	17/17
Naturally acquired low-avidity antibody only (pH test) (with few exceptions may be result of heterotypic infection)	15/19	2/3	7/7
Naturally acquired low-avidity and high-avidity antibody present	11/21	2+17/11†	6/17‡
Tested 3-4 years after 1st dose 9 to 15 months before test	14/10	1/7	17/6
No heterotypic low- or high-avidity antibody before Salk vaccine			
Received 2d dose Salk vaccine—tested 2 weeks to 3½ months after 2nd dose	9/9	6/7	9/9

\* 1/10 = 1" of 10 and actual injected virus.

† 20 and actual had a pH 1 analysis which might represent a non-

Type 1 virus at a second time 8 mo-

† = not a trace of virus

§ Note that in naturally immu-

re Type 1 and 1 out of 3 Type 3

§ attenuated virus perhaps may be

\* Attenuated feedings

§ 15 and 15

§ 15 and 15

§ 15 and 15

§ 15 and 15

Type 1 antibody to infection was no multi

resistance of to Type the same the tested by in tract

tests (13 high avidity and 13 low avidity) viruses which were fed alimentary tract

to infection which best suggested Type

§ only a few high-avidity virus and

icated in the denominator and the number who excreted virus even for the briefest period are shown in the numerator. No boost in antibody was detected in any person who failed to excrete virus. In the first group without homo-typic antibody all excreted virus except 3 adults who were fed the Type 1 virus strain. There is reason to believe that these 3 individuals may have had a previous Type 1 infection which immunized their alimentary tract but left insufficient circulating antibody to be detected by the usual tests. This is based on experimental observations.

In the naturally immune group the majority exhibited complete resistance and the few who excreted virus generally did so for only brief periods and in reduced amounts. The incidence of intestinal resistance was greater for Type 1 than for Type 3 virus. In the group immunized by ingestion of attenuated strains 8 to 15 months earlier intestinal resistance was greatest, the slight break-throughs occurring in only 3 individuals who initially experienced only a limited intestinal infection or had a simultaneous infection with more than 1 type of polio virus. In the volunteers whose antibody was induced by formalinized vaccine virus excretion was entirely comparable both in quantity and duration with that found in the first group without antibody. There is therefore some reason for assuming that an orally administered attenuated polio virus vaccine would interfere with the implantation and the dissemination of the naturally occurring virulent strains.

The real issue therefore concerns the safety of an attenuated vaccine not only as regards the viruses that are originally ingested but also of those that are excreted after varying periods of multiplication in the alimentary tract. If there were strains of polio virus completely lacking in neurotropism for the most susceptible primate neurons regardless of dose and mode of inoculation and if such strains were otherwise harmless and unlike other living organisms did not mutate there would be little difficulty in reaching a quick decision regarding their use on a large scale. Since the extensive studies of the past 4 years have failed to reveal such polio viruses the remainder of this paper will be devoted to a summary of our current knowledge about the significance of various degrees of neurotropism as it is measured quantitatively in monkeys and

of the variations in neurotropic activity of different strains before and after propagation in the alimentary tract.

The spectrum of currently available strains shown in Table 43 ranges from the most neurotropic which paralyze monkeys after intracerebral injection of 1 to 10 tissue-culture infective doses to the least neurotropic which are inactive except in a limited way in an occasional monkey after direct spinal inoculation of 1 000 000 or more tissue-culture infective doses. Strains which may be regarded as being attenuated at least 10 millionfold because 10 000 000 or more tissue-culture infective doses are inactive in most intracerebrally inoculated monkeys may still be paralytogenic intraspinally in doses of 10 to 100 tissue-culture infective doses. Thus the strains at the least neurotropic end of the spectrum represent an attenuation that is in excess of 100 millionfold but these strains still produce neuronal lesions of varying extent in a varying proportion of spinally inoculated monkeys which exhibit no paralysis. However it has also become evident from tests on large numbers of chimpanzees that polio viruses that are only partly attenuated as determined by their intracerebral and spinal activity in monkeys are not paralytogenic in maximal doses by the spinal route in chimpanzees. Some of these strains also produce no demonstrable lesions. As regards chimpanzees one can speak of a completely non-neurotropic polio virus. Since the incidence of paralysis among chimpanzees that are fed highly virulent virus is approximately 70 per cent corresponding to the maximal paralytic attack rate recorded for human beings—I am referring to the virgin soil epidemic among highly inbred Eskimos—there is at least some reason for assuming that the most susceptible human neurons are comparable to those of chimpanzees. These observations taken together with the fact that viruses recovered from the nervous system of paralytic patients invariably exhibit high intracerebral paralytogenic activity in monkeys provide us with the only criteria that we now have for estimating the potential safety for human beings of viruses in a given range of the monkey neurotropic spectrum. It is of course possible that other factors exclusive of neurotropism such as a limited ability for multiplication in extraneural tissues outside the alimentary tract



TABLE 43 NEUROTROPIC SPECTRUM OF KNOWN POLIO VIRUSES IN RELATION TO DIFFERENT PRIMATE NEURONS

Most Neurotropic 1—10 TCID Paralysis	Cynomolgus Monkeys										Least Neurotropic 10 <sup>4</sup> —10 <sup>5</sup> TCID No Paralysis			
	Brainstem Neurons							Lumbar Cord Neurons						
	10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>6</sup>	10 <sup>7</sup>	10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>6</sup>	10 <sup>7</sup>
Viruses active in this range in monkeys <i>Not paralytogenic in chimpanzees</i> in doses of 10 <sup>4</sup> —10 <sup>5</sup> TCID intraspinally														

may also influence the safety of orally administered virus

In the very first tests in chimpanzees and human volunteers it was found that after feeding partially attenuated strains the virus excreted in the stools was more neurotropic for monkeys than that which was fed. The results obtained with the stools of 7 volunteers at different times after ingestion of the least attenuated and most unstable Type 1 virus are shown in Table 44. What is shown here are the tests after ingestion of the *least* attenuated and most unstable Type 1 virus. Although the original stools of only 2 individuals exhibited some paralytogenic activity in monkeys the presence of more neurotropic particles in most of the stool specimens becomes evident when the stool is cultured for only 1 passage—and I must stress that. Too many passages give a change in the virus population. This virus had only 1 passage and larger amounts were inoculated intracerebrally. That the more neurotropic particles do not necessarily overgrow the others is evident in the tests on volunteer Mal whose 9-day stool exhibited increased neurotropic activity while that obtained at 56 days and 140 days—and this is the longest carrier state observed in our study—was inactive at the 100 000 TCID level. The most active stool culture in this series that of volunteer Har failed to produce paralysis in 3 chimpanzees inoculated intraspinally with more than 1 000 000 tissue culture infective doses of virus. Similar negative results with 2 other strains were obtained in 6 chimpanzees inoculated with stool cultures of similar intracerebral neurotropic activity in monkeys. Thus there is at least some reason for assuming that the increase in monkey neurotrop-

ism observed in these tests is not necessarily in the harmful range for human beings. Although this particular Type 1 strain was unstable even on propagation in vitro similar although less frequent and less extensive increases in neurotropicism were observed with 4 naturally occurring attenuated Type 1 strains. This is quite a general property. It also became evident that the extent of increase in neurotropicism after multiplication in the alimentary tract might be related to the degree of attenuation of the virus that was fed. The results of tests on early and late stools of 5 volunteers who were fed the highly attenuated Type 1 L5c strain (Table 45) yielded no paralysis in any of the 42 monkeys inoculated intracerebrally with amounts of virus up to 800 000 tissue-culture infective doses. The reason some of the original stools were not tested is that they contained too little virus to be of significance. The stools of 3 chimpanzees that ingested large amounts of this strain yielded similar results not only in intracerebrally but also in spinally inoculated monkeys. However the results of spinal tests in monkeys with the stool cultures of 6 volunteers indicated an increase in neurotropic activity in this higher range of the spectrum in at least 3 individuals. Nevertheless these were the best results obtained with any of the Type 1 strains tested and therefore this strain was selected for further study.

Essentially similar results were obtained with the Type 2 and the Type 3 polio viruses and the strains selected for further study exhibited the least neurotropicism after multiplication in the alimentary tract.

Since all the strains tested up to this point had been purified only by the terminal dilution

TABLE 44 INTRACEREBRAL ACTIVITY IN CYNOMOLGUS MONKEYS OF VIRUS EXCRETED IN STOOLS OF HUMAN VOLUNTEERS FED LEAST ATTENUATED AND MOST UNSTABLE TYPE I VIRUS (MAHONEY KP 33)

VOLUNTEER	DAYS AFTER INGESTION OF VIRUS	ORIGINAL STOOL		STOOL CULTURE	
		TCD <sub>50</sub> INOCULATED LOG 10	RESULT	TCD <sub>50</sub> INOCULATED LOG 10	RESULT
Wal	6	3.2	0 0	5.2	0 0 11
Hac	7	4.2	0 0	5.9	0 8 9
Ric	10	3.2	0 0	5.7	0 9 9
Har	10	4.2	0 8 23	4.9	8 8 1
Car	14	2.5	0 0	4.9	0 7 10
Tro	14	5.0	0 0 0	4.9	0 0 0
Tro	28	3.2	0 0 0	5.9	0 8 9
Mal	9	3.2	0 0 15	5.4	0 8 10
Mal	56	3.2	0 0 0	5.7	0 0 0
Mal	140	—	—	4.9	0 0 0 0
Total		2.5-5.0	3/23	4.9-5.9	14/31

11 = incubation period (days) of paralytic monkey 0 = no paralysis

Note: Cult. fluid fed to lunate w. ot paralytic gen. c. f. monkey inoculated intracerebrally with ml of reagent but subsequent passage produced from it usually paralytic agent per port. n. Intra. fly. th. ml of d. s. were passy. k. n. f. r. cyn. m. lgu. m. key. b. e. n. th. l. eg. r. m. l. d. w. r. paralytic gen. n. h. mp.

TABLE 45 INTRACEREBRAL ACTIVITY IN CYNOMOLGUS MONKEYS OF VIRUS EXCRETED IN STOOLS OF HUMAN VOLUNTEERS FED MOST HIGHLY ATTENUATED TYPE I VIRUS PURIFIED ONLY BY TERMINAL DILUTION TECHNIC (LSC)

VOLUNTEER	DAYS AFTER INGESTION OF VIRUS	ORIGINAL STOOL		STOOL CULTURE	
		TCD <sub>50</sub> INOCULATED LOG 10	RESULT	TCD <sub>50</sub> INOCULATED LOG 11	RESULT
Moo	7	3.2	0 0 0	4.9	0 0 0
Moo	11	—	—	5.9	0 0 0
Kut	7	3.2	0 0 0	4.7	0 0 0
Kut	21	—	—	5.9	0 0 0
Mar	7	3.2	0 0 0	4.7	0 0 0
Mar	21	—	—	4.2	0 0 0
Mc	7	2.5	0 0 0	5.2	0 0 0
Mc	14	—	—	5.7	0 0 0
Ree	7	—	—	5.2	0 0 0
Ree	14	—	—	5.2	0 0 0
Total		2.5-3.2	0/12	4.2-5.9	0/30

Note: Cult. fluid fed and 1 equivalent passages from it in 11 monkeys in not acutely inoculated monkey generated 1111 l.d.s.

TABLE 46 INTRACEREBRAL ACTIVITY IN CYNOMOLGUS MONKEYS OF OPTIMUM SINGLE PLAQUE STRAINS OF EACH TYPE OF POLIO VIRUS GROWN IN 20 TO 25 LITER LOTS OF MONKEY KIDNEY TISSUE CULTURE

TYPE	STRAIN	PFU VIRUS PER ML.	PARALYTOGENIC EFFECT OF 1 ML. OF INDICATED DILUTION	
			UNDILUTED	10:1
1	LSc 2 ab	$4.2 \times 10^7$	0/10	0/5
2	P 712 Ch 2 ab	$3.6 \times 10^7$	0/10	0/5
3	Leon 12 a b	$4.3 \times 10^7$	0/10	0/5

Not Intracerebral inoculation of  $10^7$  PFU or more of other lots of these strains in groups of 10 monkeys also yielded negative results. Intramuscular (deltoid) injection of about  $10^5$  PFU or more (2 ml. undiluted culture fluid) of each strain in groups of 10 to 15 cynomolgus monkeys was also without paralytogenic effect.

technic in culture tubes and may have represented progeny originally derived from more than 1 virus particle. It was possible that any increase in neurotropic activity of virus excreted in the stools may have been due to the plating out in the alimentary tract of a pre-existing mixed population of virus particles in the culture fluid that was fed. When the populations of the 3 optimum strains were dissected so to speak by obtaining the progeny of many single triply purified plaques, it was indeed found by quantitative spinal tests in monkeys that the virus particles making up each strain were not homogeneous in their neurotropic activity; some exhibited approximately the same spinal activity as the total population making up the original strain, others were distinctly more neurotropic and 1 or 2 among 9 or 10 particles tested from each strain exhibited the least neurotropic activity that had been encountered thus far. Since repeated *in vitro* subcultures of these highly attenuated single plaque derivatives yielded similar results on spinal inoculation in monkeys, it became possible to test in a definitive way the difference in the viral populations produced by many cycles of multiplication in the alimentary tract from those produced during the relatively few cycles of multiplication in tissue cultures *in vitro*.

The progeny of 37 separate triply purified plaques derived from 6 different Type 1 strains were tested by spinal inoculation in monkeys before selecting the optimum Type 1 strain. Although a few other plaques yielded virus that was as highly attenuated, none was superior to it. The Type 2 single plaque strain that was ultimately

selected was derived from a stool culture of a chimpanzee that had been fed the optimum single plaque virus segregated from the P 712 strain—a naturally occurring strain. The Type 3 strain is derived from the optimum plaque segregated from the attenuated Leon virus. Approximately 25 liter lots of each of these strains then were grown in rhesus kidney cultures with the generous co-operation of the Merck, Sharp and Dohme Research Laboratories. After Seitz filtration and appropriate tests in monkeys, chimpanzees, rabbits, guinea pigs, mice and tissue cultures, aliquots of these lots were fed to 10 adult volunteers. The standard dose, based on previous observations in human beings, was 0.01 ml. of culture fluid—approximately 100,000 plaque forming units—and this was given in a tea spoonful of cherry syrup. Many stool specimens from each of these individuals were tested in tissue culture and no agents other than the ingested polio viruses were recovered.

In order to be able to evaluate the significance of the tests for neurotropic activity of the virus found at different times in the stools of these volunteers, it is necessary to examine the data on the viruses that were fed.

Table 46 shows the tests for intracerebral and intraspinal activity in cynomolgus monkeys of the large lots of each type of virus used in these tests. Completely negative results were obtained in these as well as in preceding intracerebral tests even when as much as 40,000,000 plaque forming units were inoculated in large numbers of monkeys. The spinal tests show that only an occasional monkey exhibited a slight localized or questionable effect after inoculation of very

TABLE 47 INTRACEREBRAL PARALYTOGENIC ACTIVITY IN MONKEYS OF VIRUS CULTURED FROM STOOLS OF VOLUNTEERS FED OPTIMUM TYPE 1 SINGLE PLAQUE STRAIN (LSc 2 AB)

STOOL CULTURES FROM INDICATED NUMBER OF VOLUNTEERS OR INDIVIDUAL VOLUNTEER	DAYS AFTER INGESTION OF VIRUS	TCD <sub>50</sub> INOCULATED LOG 10	RESULT
20	6-7	6.7-7.9	1/80
"	14	7.0-7.4	0/8
4	21	7.0-7.4	1/16
Bl	28	6.9	0/4
Hou	28	7.7	1/4
		6.7	1/4
		5.7	0/4

The monkey exhibited paralysis from 22 days after inoculation but did not develop any paralysis. The virus was recovered from the spinal cord.

Volunteer Hou was inoculated 45 months later.

large doses. Doses in the range of 100 000 000 plaque forming units inoculated in the deltoid muscles of large numbers of cynomolgus monkeys were also without effect.

Table 47 shows the results of intracerebral monkey tests on the stool cultures of 20 volunteers at 6 to 7 days after ingestion and on 8 stool cultures obtained at 14 to 28 days the longest period of excretion in this group of volunteers. It should be noted that virus multiplication in the alimentary tract begins within 24 to 48 hours after ingestion and that at 6 to 7 days the virus in the stools already represents the progeny of many cycles of multiplication. Among the 80 monkeys inoculated with 5 000 000 to 80 000 000 TCD<sub>50</sub> of virus from the 6- to 7-day stool cultures only 1 exhibited slight paralysis at 22 days after inoculation and no cytopathogenic virus was found in its spinal cord. However 2 of the 8 monkeys inoculated with 5 000 000 or 50 000 000 TCD<sub>50</sub> of the 28-day stool culture of 1 volunteer exhibited paralysis indicating that virus in this particular stool had a slightly greater neurotropism than the virus that was fed. This interpretation is confirmed by the results of the spinal tests in monkeys shown in Table 48. The 28-day stool culture of volunteer Hou shows a distinct increase in spinal activity over that found in his 7-day stool culture and in the 14 to 28-day stool cultures of the other 7 volunteers tested.

The same dose of this Type 1 virus was also fed to 5 children who were 5 to 11 years of age

TABLE 48 SPINAL ACTIVITY IN MONKEYS OF VIRUS CULTURED FROM STOOLS OF VOLUNTEERS FED OPTIMUM TYPE 1 SINGLE PLAQUE STRAIN (LSc 2 AB)

VOLUNTEER	DAYS AFTER INGESTION OF VIRUS	TCD <sub>50</sub> INOCULATED LOG 10	RESULT
Hou	7	5.4	1/4
	28	6.0	4/4
		5.0	2/4
		4.0	3/4
Bl	7	5.4	2/4
	28	5.2	1/4
Bu	14	5.7	0/4
Mil	14	5.7	1/4
Cit	21	5.7	0/4
Cris	21	5.7	0/3
Hoc	21	5.7	2/4
Mar	21	6.0	2/3

and possessed no antibody for any of the 3 types of polio virus. Two stool cultures from each child obtained 5 to 10 and 17 to 21 days after ingestion of virus were tested in a total of 40 intracerebrally inoculated monkeys with results similar to those shown for adults in Table 47. Here also the spinal tests revealed an increase in neurotropic activity.

Table 49 shows the results of intracerebral tests in monkeys with 20 stool cultures. One cannot get anywhere by testing a few stools of a few individuals. Of course if one has a fairly attenuated strain it is satisfactory. But with

ministration the response is so fast even in those without previous exposure that there seems to be no difference. However, a recent boost in antibody by formalinized vaccine has been found to interfere with the boosting capacity of an alimentary infection. Dr Salk aided us in some of this work. We carried out the tests together. Similarly a dose of killed virus vaccine may be without effect when it is administered intramuscularly at too short an interval following a previous dose. When live virus is given by mouth to individuals who previously had killed virus vaccine an interval of at least 3 months would appear to be desirable.

No strains that can multiply elsewhere in the body but not in the alimentary tract have yet been found. In fact my earliest studies have shown the reverse namely that intramuscular injection of an attenuated strain may be ineffective except when it localizes in the alimentary tract. While the results of the present study clearly indicated that relatively more neurotropic mutants can occasionally arise in the alimentary tract they also established that when highly attenuated strains are fed the resulting mutants are still in that attenuated range of the neurotropic spectrum in which a million or more tissue-culture infective doses are harmless even after direct inoculation in the spinal cord of chimpanzees. Therefore they may be expected to be harmless for man.

Our own previous studies in 133 volunteers and the tests of Koprowski and of Dick on many hundreds of individuals with strains of different degrees of attenuation as well as our present tests in 110 individuals with the very highly attenuated strains were all without any harmful effects. However it would probably require tests on tens of thousands of people to establish whether the theoretical expectations of safety are confirmed in actual practice. Simultaneous feeding of such a vaccine to entire families and communities during seasons of the year when polio viruses naturally disseminate poorly or in communities where polio viruses of varying degrees of virulence are known to be spreading extensively already might be the best way to conduct such tests. But where and under what circumstances would tests on increasingly larger numbers of people be justified? The definite reduction in the incidence of paralytic polio-

myelitis achieved thus far by killed virus vaccine may naturally incline to a decision that in countries where mass application of killed virus vaccine is feasible it be given an opportunity to show what can be achieved over a period of years. If the passage of time should prove that immunity resulting from the killed virus vaccine supplemented in at least some individuals by natural infection is indeed long lasting there might never be any need for considering the use of a live virus vaccine. If however time should prove that the immunity conferred by a killed virus vaccine is of relatively short duration in a large proportion of individuals then consideration might be given to supplementation of the waning immunity by feeding of the best available attenuated vaccine a procedure that may be expected not only to reinforce the humoral immunity but also to induce that resistance of the intestinal tract to reinfection which could provide the only means for the possible eradication of poliomyelitis.

On the other hand in countries where mass application of a killed virus vaccine is not feasible and where polio viruses of varying degrees of virulence are already known to be undergoing extensive spread in the population there would appear to be sufficient justification for initiating at this time trials of the currently available tested lots of highly attenuated polio virus vaccine. And such trials are now actually in progress. Finally I should like to say that it is obvious—at least so it seems to me—that more work, time and experience ultimately will teach us the best way to eliminate poliomyelitis as a threat to human well being.

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# *Epidemiology of Poliomyelitis in Populations Before and After Vaccination with Inactivated Viruses*

DR JOHN P FOX\*

Observations of the occurrence of poliomyelitis and more recently of past and current infection as distinct from disease have led to a reasonable hypothesis as to the epidemiology of poliomyelitis. Although not all aspects are accepted universally a brief summary is an essential introduction to the work to be described. This hypothesis should explain such phenomena as (1) the relatively recent emergence of poliomyelitis as an epidemic disease (2) the relative restriction of epidemic disease to the more highly developed portions of the temperate zones of the earth (3) the original characteristic age pattern and the shifts in that pattern toward older age groups (4) certain correlations between various socioeconomic factors and both infection and disease and (5) the relative infrequency with which infection results in paralytic disease.

Etiologically we recognize a family of small hardy viral agents composed of at least 3 distinct but antigenically related members which apparently exist in nature only in relation to man. While respiratory transmission may occur the oral portal of entry is usually postulated with consequent infection of the alimentary tract and reasonably prolonged shedding of the virus in the feces. Variations in the age pattern of infections are explained best in terms of variations in the specific pathways predominantly followed by fecal virus in reaching the oral portal. Utilization of the numerous possible indirect pathways i.e. polluted food and water and flies is related inversely to the level of environmental sanitation and probably predominates in underdeveloped areas. In areas of adequate environmental sanitation personal contact among the usually susceptible and less hygienic young-child segment of the population is probably the dominant mechanism. Significant neurologic disease as a consequence of infection is determined by the

operation of numerous factors including strain virulence, host age and such predisposing host factors as fatigue, recent inoculations, pregnancy and absence of tonsils. Increasingly since about 1880 the originally tolerable balance between polio viruses and the human host has been disturbed, probably chiefly by influences operating to delay infection to an age when it is apt to result in disease. However it still remains true that relatively few infections result in significant disease.

With the tremendous technical advances resulting from the adaptation of tissue-culture methods to polio virus cultivation<sup>6, 7, 8, 9, 10</sup> prospective studies of the natural process of infection without reference to disease became feasible. That to be described was initiated in southern Louisiana in 1953 with the hope that it would permit new insight into such diverse problems as defining the exact mechanisms of virus transmission and those factors which determine that a particular infection will result in paralytic disease; the significance of postinfection immunity with reference to duration and influence on both homologous and heterologous reinfection and the phenomenon of the seasonal occurrence of disease. The observations centered about a stable group of households recruited to be reasonably representative with respect to residence, family size, race and economic status. Well before all of the questions posed had been answered it became necessary to offer the study group the benefits of artificial immunization. With a primary 2-dose course of Salk vaccine completed during January 1956 the study was reoriented toward determining the influence of vaccine-induced immunity on the natural occurrence of alimentary tract infection and viral dissemination. In January 1957 the third or booster dose of vaccine was administered and the observations have continued with the same orientation.

\*Written in collaboration with Dr. Henry M. G. Leland, Dr. Dorothy R. LeBlanc and Dr. Dwight F. R. W.

The purpose of the present paper is to review briefly the previously reported observations in the period 1953 to 1955 prior to vaccination<sup>8,9,10,11</sup> which have been reported only partially elsewhere<sup>12</sup> and to report briefly on observations made in the first few months of 1957 after the booster dose of vaccine.

### FIELD AND LABORATORY METHODS

The most important single facility was the study group itself. This originally comprised 157 households recruited in 1953 having in common 2 essential attributes: (1) a newborn child to serve as the known nonimmune index individual and (2) a high probability of co-operation continuing through several years of observation. Otherwise they were divided as equally as possible into a number of subgroups defined on the basis of family size, area of residence, race and within the white group economic status. Three study areas were established: these consisted of urban New Orleans with a relatively low prior poliomyelitis morbidity (8 per 100,000 population in 1950-1954), urban Baton Rouge with a high prior morbidity (29.9) and 4 semirural parishes in south-central Louisiana of low past incidence (13.1) designated as the Evangeline Area. As vaccination was offered in January 1956, there remained 136 households containing 139 original index children, 675 older associates and 76 younger siblings. With the reorientation of the study, 18 families withdrew leaving 118 under observation after vaccination. During 1956 an additional 8 families were lost. However, 9 new families each closely related to an original study household were added so that the 1957 observations began with a total of 119 households.

On admission of each household, a basic record was opened which contained pertinent current and historical information. Each household member was bled on admission to the study and annually thereafter in order to permit detection of possible episodes of infection which had not involved the index child. At monthly intervals continuing through 1955, blood and stool specimens were collected from the index child and the entire household. Following vaccination in 1956, routine bleedings were made only in relation to vaccination, but stools were collected

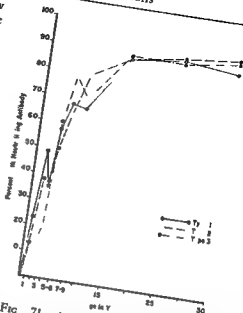


FIG. 71. Age specific percentages of household associates of index children with neutralizing antibodies to Types 1, 2, and 3 polio viruses when admitted to the study in 1953 (Fox, J. P. *et al.* *Am J Hyg.* 65:344).

twice monthly from all children under 15 years of age. In all cases, detection of infection of any child led to a special visit for added information and for specimens from household and other indicated associates.

The laboratory methods have been described in detail elsewhere.<sup>10</sup> Although changes were made as improved methods were developed, the basic observations were derived from the use of monolayer film cultures of trypsin-dispersed monkey kidney cells.<sup>3</sup> This applies not only to virus isolations but also to tests for neutralizing antibody. This latter point is of importance since according to Sabin,<sup>6</sup> this method reveals only high avidity antibody. With the exception of those specimens obtained from young children by heel or finger puncture which could not be tested in final dilution lower than 1:10, all statements as to absence of antibody are based on testing sera in final dilution of 1:2.

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Although the study group constitutes but a small sample of the total population and is repre-



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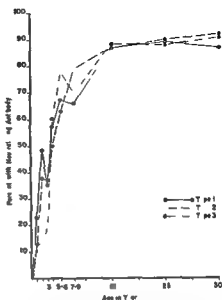


FIG. 71. Age specific percentages of household associates of index children with neutralizing antibodies to Types 1, 2 and 3 polio viruses when admitted to the study in 1953 (Fox, J. P. *et al.* *Am. J. Hyg.* 65: 344).

twice monthly from all children under 15 years of age. In all cases, detection of infection of any child led to a special visit for added information and for specimens from household and other indicated associates.

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### PATTERNS OF SERO-IMMUNITY IN THE STUDY GROUP

Although the study group constitutes but a small sample of the total population and is repre-

TABLE 56 DISTRIBUTION OF EPISODES OF HOUSEHOLD INFECTION IN SOUTHERN LOUISIANA BY MONTH AND BY VIRUS TYPE IN 1954-1955 BEFORE VACCINATION AND IN 1956 AFTER VACCINATION

YEAR	VIRUS TYPE	NUMBER OF EPISODES IN MONTH INDICATED												TOTAL
		J	F	M	A	M	J	J	A	S	O	N	II	
1954	1								II	4	1		1	8
	2						4	10	2		2	1	1	20
	3			1		3	3	9	2	3	7	1		29
	All			1		3	7	19	6	7	10	2	2	57
1955	1	2		1	4	2	2	6	6	1			1	25
	2	1		2		1	2	2	3	2		1	1	15
	3	2							3			2	1	8
	All	5		3	4	3	4	8	12	3		3	3	48
1956	1		2	2	2	4	4	3	5	2	5			29
	2				1	1	2							4
	3				1		5	5	3					14
	All		2	2	4	5	11	8	8	2	5			47
1954-1956 All		5	2	6	8	11	22	35	26	12	15	5	5	151

TABLE 57 POLIO VIRUS TYPES ISOLATED FROM REPORTED CASES OF POLIOMYELITIS AND FROM NONCLINICAL INFECTIONS IN PRIMARY INDEX CHILDREN SOUTHERN LOUISIANA 1954-1955\*

TYPE OF POLIO VIRUS	ISOLATIONS IN 1954				ISOLATIONS IN 1955				TOTAL ISOLATIONS			
	CASES		INDEX INFECTIONS		CASES		INDEX INFECTIONS		CASES		INDEX INFECTIONS	
	No	%	No	%	No	%	No	%	No	%	No	%
1	II	35	8	14	24	50	25	52	30	46	33	37
2	3	18	20	35	4	8	15	31	7	11	35	33
3	8	47	29	51	20	42	8	17	28	43	37	35
TOTAL	17	100	57	100	48	100	48	100	65	100	105	100

Gelfand H M *et al* Am J Hyg 65:367 1957

viruses and looking at the 1956 data perhaps of Type 2 virus as well. However it should be pointed out that the distribution by type of viruses isolated from clinically manifest infections occurring in the study areas did not parallel that of the viruses isolated from the household episodes. This is shown in Table 57. Type 1 virus in 1954 and Type 3 virus in 1955 were

disproportionately common among the instances of disease. In effect this suggests that among the more virulent strains Types 1 and 3 predominated in 1954 and 1955 respectively.

Distribution of the infections in index children by their age in months revealed no obvious age of greater susceptibility. The earliest infection was detected as of 23 days of age and a

TABLE 58 INFECTIONS AMONG ASSOCIATES OF INFECTED INDEX CHILDREN IN 1954-1955 PRIOR TO VACCINATION IN RELATION TO HOMOLOGOUS IMMUNITY STATUS PRIOR TO HOUSEHOLD INFECTION EPISODE

PRIOR IMMUNITY	STATUS IN RELATION TO INDEX CHILD	SEROLOGIC RESPONSE TO INFECTION	NUMBER OF PERSONS		PROVED INFECTIONS	
			TOTAL	EXCEPT NO. VIRUS	RATIO	%
None	Adult	None	4	0	1:15	73
		Yes	11 (7)†	3		
	Sibling	None	8	0	1:25	94
		Yes	125 (77)	54		
	All Associates	None	12	0	1:36	148
		Yes	136 (104)	57		
Yes	Adult	None	179 (115)	3	27:703	13
		Yes	24 (16)	11		
	Sibling	None	64 (44)	1	38:101	38
		Yes	37 (30)	3		
	All Associates	None	243 (152)	4	65:304	1
		Yes	61 (46)	3		

Ratio of number of persons of each age group exposed

† Figure in parentheses indicates number of persons from whom the age-specific percentages were calculated in the first period of the infection of the household

total of 20 occurred during the first 6 months of life 4 of these in the demonstrated presence of homologous maternally derived antibody. Family size and race or economic status were of importance infections being more frequent in one or both of the years 1954 and 1955 among index children with 2 or more older siblings and among those in Negro or lower-economic white families. In this connection it is interesting to note that Melnick and co-workers<sup>21</sup> in the prospective studies in Arizona and West Virginia were able to relate within the lower economic groups both family size and level of household sanitation to the frequency of both past and observed infections.

The increased risk related to having older siblings together with a number of isolated observations suggests that an older but preschool sibling often brings the virus into the home. Once in the home as indicated in Table 58, virus typically spreads to the other homologously nonimmune household members (97%) and provides a challenge exposure which frequently

results in the reinfection of those already immune (at least 21% overall). However as Table 58 suggests focal virus was much less readily detected in the single specimens collected from persons undergoing reinfection (7% of 65 or about 10%) than in those from persons with primary infection (5% of 136 or about 4%). Suggestive evidence also was obtained for spread between adjacent households and within a neighborhood. Interestingly this observation could not be confirmed by Melnick and his group<sup>22</sup> in the study of the upper-economic group in Charleston West Virginia. Only once were we able to link an episode with some certainty to an instance of paralytic disease in the neighborhood.

Including associates as well as index children there were 244 instances of primary infection at an approximately known time. Illnesses taken shortly after the infection episodes were detected revealed no illness in relation to 73 per cent of the infections and only minor febrile illness in 74 per cent. In 9 instances (4%) aseptic men-

ingitis was suggested in retrospect. In no instance did paralytic disease result. To emphasize further the relative safety of natural immunization under the conditions prevailing in southern Louisiana we may mention our computation based on infection rates in our study group as applied to the general population of the area that about 850 infections were required to produce 1 case of paralytic disease.

### INFECTIONS IN THE STUDY GROUP DURING 1956

Observations in 1956 after primary vaccination were modified to permit more certain detection of alimentary tract infections in that stools were collected twice monthly from all children under 15 years of age rather than once monthly from the index child.

All vaccinations were with a single unselected lot of commercially produced vaccine. The seroimmunity status of the group 1 month after the second dose of vaccine is shown in Table 59. Of the 118 children with no antibody before vaccination 36.26 and 60 per cent failed to develop antibodies capable of inhibiting the cytopathic effects of Types 1, 2 and 3 viruses respectively. However, much better *de novo* development of antibody was observed in those already possessing naturally acquired antibody to 1 or 2 heterologous virus types.

As noted in Table 56 there were 47 episodes of household infection in 1956 among the 118 households under observation. Two of these were limited to single individuals undergoing reinfection and would not have been detected by the methods of observation previously employed. The 45 remaining episodes all involved one or more persons experiencing primary infection and may be compared with 52 episodes observed in 1955 prior to vaccination among about 140 households. Evidently primary vaccination did not act to reduce the frequency of household episodes.

Information regarding virus spread within 43 households is shown in Table 60 and should be compared with the data contained in Table 58 but with two important reservations. First, the 1953-1955 data in Table 58 relate only to households in which the episodes involved the index child; comparable data for the 10 abortive episodes in which the index child escaped being incomplete. If such data could have been included the

TABLE 59. NEUTRALIZING ANTIBODY DEVELOPING *DE NOVO*\* 1 MONTH AFTER 2 DOSES OF SALK VACCINE IN RELATION TO SERO-IMMUNE STATUS PRIOR TO VACCINATION

PRE VACCINATION IMMUNITY	POSTVACCINATION IMMUNITY NUMBER OF PERSONS AND PER CENT WITH TITERS OF 1% AGAINST VIRUS				
	No	0†	2-5	10-70	40+
TYPE 1					
None	118	36 (35)	30	28	6
Only Heterologous	97	10 (6)	22	41	27
TYPE 2					
None	118	26	21	41	12
Only Heterologous	97	8 (3)	14	39	38
TYPE 3					
None	118	60 (59)	23	13	5
Only Heterologous	94	18 (13)	37	35	15

\* These data refer only to development of antibody of the type or type as the parent prior to vaccination.  
† The percentage classed as negative include specimens negative in 1:2 dilution; the separate figures for which are shown in parentheses and these include or exclude pure specimens which were negative in 1:10 dilution which was the lowest that could be tested.

resulting figures for spread of virus within all households infected prior to vaccination probably would have been very close to those shown in Table 60 for the postvaccination experience (87 primary infections and 16% reinfections disregarding age). Second, due to the boosting effect of vaccination on pre-existing antibody levels it is even more probable in 1956 than in 1954-1955 that only a portion of the actual reinfections were detected serologically because of the difficulty in showing a significant antibody rise in persons whose titers already are high. In any event the reinfections detected in 1956 are noteworthy because in 10 instances they occurred despite presumed reinforcement of the natural immunity by vaccination. Over all it does not

TABLE 60 VIRAL EXCRETION AND SEROLOGIC RESPONSE TO INFECTION AMONG MEMBERS OF 43\* HOUSEHOLDS UNDERGOING EPISODES OF POLIO VIRUS INFECTION IN 1956 AFTER A PRIMARY COURSE OF SALK VACCINE

HOMOLOGOUS PREVACCINATION IMMUNITY	AGE GROUP (YEARS)	SEROLOGIC RESPONSE TO INFECTION EPISODE	NUMBER OF PERSONS		PROVED INFECTIONS	
			TOTAL	EXCRETING VIRUS†	RATIO‡	%
None	15 and over	None	4	0	1/6	33
		Yes	2	1		
	Under 15	None	12	3	87/70	91
		Yes	84	81		
	All ages	None	16	3	89/100	87
		Yes	86	83		
Yes	15 and over	None	80	0	8/88	9
		Yes	8	2		
	Under 15	None	40	6	11/45	29
		Yes	7	5		
	All ages	None	120	6	21/133	16
		Yes	15	7		

\* Four household episodes excluded because data incomplete (2 household) single individual infected (2 household) was experiencing infection and/or than primary infection.  
 † Data as to virus excretion based on per cent collection of virus in the urine of persons under 15 years of age whereas in case of older persons they depend on single specimens taken soon after infection of young persons was detected.  
 ‡ Ratio of number of persons infected to number exposed.

TABLE 61 DURATION OF FECAL EXCRETION OF VIRUS IN CHILDREN AS RELATED TO HOMOLOGOUS IMMUNITY STATUS PRIOR TO INFECTION

PREVIOUS NATURAL IMMUNITY	VACCINATION IMMUNITY		NUMBER OF CHILDREN EXCRETING VIRUS	DAYS DURATION OF VIRUS EXCRETION		
	VACCINE	SERO-RESPONSE		OBSERVED		ESTIMATED TRUE MEAN
				RANGE	MEAN	
None	None	None	110†	1-114	24	51
	Primary Only	None	34	1-105	23	47
		Yes	50	1-85	24	47
	All with primary		84	1-105	26	44
Present	Yes‡	Yes & No	15§	1-43	7	22

As detected by cytopathic rather than pH test.

† Infections occurring in 1954-1955 prior to vaccination.

‡ All but 2 had been vaccinated.

§ All but 4 members of the households not included in Table 60.

seem that vaccination influenced intrahousehold spread of infection

A final aspect of interest is related to excretion of virus in the feces. Of the 87 children under going primary infection after vaccination only 3 were not found to be excreting virus. As Table 60 further indicates when continuous observation was maintained viral excretion also was commonly found among children who were experiencing reinfection. This was the case in 5 of the 7 manifesting antibody rise and in 10 additional children (including 4 not mentioned in Table 60). The data in Table 61 indicate that duration of excretion was significantly reduced in children undergoing reinfection whereas in children experiencing primary infection it did not differ materially as between unvaccinated and vaccinated children or within the vaccinated group as between those who did or did not manifest homologous serologic response to vaccination. Finally in Table 62 we have a crude estimate of the levels of fecal infectivity in the first virus positive specimens. Not only was there no significant difference related to vaccination in the specimens from primary infections but also rather surprisingly nearly equal fecal infectivity was found in reinfections. In summary the primary course of vaccination did not appear to influence either the frequency the duration or the amount of fecal virus excretion in primary infections. In contrast prior natural immunity did appear to shorten the period of excretion in reinfections.

#### PRELIMINARY REPORT OF OBSERVATIONS IN 1957 AFTER THE BOOSTER INOCULATION OF VACCINE

The third or booster dose of vaccine was given during January 1957 and bloods were collected 1 month later. In Table 63 are presented the

data as to neutralizing antibody attributable only to vaccination in the postbooster sera of those children not exposed to intrahousehold infection in the interval since primary vaccination. Special interest pertains to those who were completely nonimmune prior to vaccination and failed to respond detectably to the primary course. Unfortunately a significant proportion of specimens were obtained by heel or finger puncture and could not be tested in dilution lower than 1:10. The Type 2 response was again the best titers of 1:10 or higher being elicited in all cases and of 1:160 or better in 89 per cent. The response to Type 1 was intermediate and that to Type 3 much the poorest the percentages negative in 1:2 or 1:10 dilution being 23 and 50 respectively. Apparently few of those who failed to develop Type 1 or Type 3 antibody after primary vaccination were conditioned to respond to the booster inoculum with antibody detectable in 1:10 dilution in spite of the fact that the booster was from a commercially prepared lot of vaccine selected with the advice of the Division of Biologic Standards as being of maximal potency. However a very different picture is seen among those who possessed 1 or 2 types of naturally acquired antibody prior to vaccination. With but 2 exceptions (both in respect to Type 3 antigen) all responded and very commonly with titers of 1:160 or higher.

Data as to infections in the study group during 1957 are complete only for the first 4½ months. Over all enteric viral infections were detected frequently with 42 Coxsackie 29 ECHO and 15 unclassifiable viruses but only 6 polio virus infections. These latter represented 1 household episode each of infections with Types 1 and 3 viruses. The small number of episodes at this stage in the year has little mean

TABLE 62. INFECTIVITY OF THE INITIAL VIRUS POSITIVE STOOL SPECIMENS IN RELATION TO PREINFECTION IMMUNITY STATUS OF THE DONORS

PRIOR NATURAL	VACCINATION	NUMBER OF PERSONS	LOG INFECTIVITY TITER*		
			MEAN	MEDIAN	RANGE
None	None	15	4.9	5.0	<2.0-6.5
	Yes	43	4.8	5.0	<2.0-6.5
Yes	Yes + No	11	4.2	5.0	<2.0-5.5

\* Titer expressed as log TCD<sub>50</sub> per Gm of stool and as d determined very roughly based on use of 2 culture tubes per tenfold serial dilution. It should be noted that, since large inoculum to each tube was 0.1 ml of 10 per cent extract of stool the lowest log titer detectable is 2.0 log<sub>10</sub> per Gm of original stool.

TABLE 63 OBSERVATIONS 1 MONTH AFTER THE THIRD OR BOOSTER INOCULUM OF SALK VACCINE AS TO NEUTRALIZING ANTIBODY TO POLIO VIRUSES ATTRIBUTABLE SOLELY TO THE VACCINE\*

IMMUNITY PRIOR TO PRIMARY VACCINATION	NUMBER OF PERSONS	PER CENT OF PERSONS WITH ANTIBODY TO VIRUS TYPE INDICATED IN TITERS OF 1 X																			
		Type 1									Type 2									Type 3	
		0†	2-5	10-20	40-80	160-320	640+	0†	2-5	10-20	40-80	160-320	640+	0†	2-5	10-20	40-80	160-320	640+		
None	62	23 (8)	5	10	18	27	16	0	0	3	8	55	34	50 (16)	6	13	18	8	5		
Only Type 1	19																				
Only Type 2	14	0	0	7	7	43	43	0	0	10	10	53	26	0	10	16	37	21	16		
Only Type 3	26	0	0	8	4	23	65	0	0	4	8	38	50	7	0	29	7	43	14		
2 heterologous types	10 16 10 0	0	0	10	0	60	30	0	0	0	31	63	6	5	5	10	25	35	20		
These data refer only to the development of antibody of the type or types in the household during the 11 months interval in 1956 between completion of primary vaccination and the household survey.																					
† As in Table 59																					

\* These data refer only to the development of antibody if the type or type in titer sent prior to the primary course of vaccination in person who were not exposed to infection within the household during the 11 months interval in 1956 between completion of the primary course and administration of the booster inoculum.

† As in Table 59.



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## DISCUSSION

DR. BROWN: I do not intend to enter into any discussion of the relative merits of live or killed virus vaccines. Our primary objective is the control of poliomyelitis by whatever method or combination of methods proves to be most satisfactory.

Dr. Fox has made a beautiful study and one that represents another demonstration of the wholehearted and unselfish co-operation on the part of the test subjects themselves in furnishing specimens over such a long period of time.

The work of Dr. Sabin is indeed a perfect example of precise thorough and exacting research and demonstrates the extent of attenuation obtained with single plaque strains of virus.

Dr. Koprowski in his interesting paper shows clearly that persons previously vaccinated with inactivated virus respond to the feeding of attenuated virus with marked antibody production. The increased titers obtained were in fact as high as those of some of the persons previously exposed to attenuated virus and fed live virus again after 2½ years.

Both Koprowski and Sabin have emphasized the point that vaccination with living virus does seem to prevent the development of a carrier state on re-exposure while previous vaccination with killed virus vaccines does not. In the latter instance therefore virus is being recirculated in the community. But we must not forget that the vaccinated subjects are protected against paralysis and that more and more individuals

are having their immunity re-enforced as a response to the ensuing subclinical infections.

Two of the most pertinent questions to be answered are (1) What is the duration of immunity following the administration of killed virus vaccines? and (2) What level of circulating antibodies is necessary for protection against paralysis?

Dr. Koprowski has shown that antibodies may persist for 3 to 6 years following administration of attenuated viruses. This is valuable information. Although Dr. Salk has previously described studies of the duration of antibodies following inactivated vaccines I present the results of the study which we have made in children receiving commercial inactivated vaccines under actual field conditions.

Figure 76 presents the geometric mean antibody titers of a group of school children before and after primary vaccination during the nation-wide field trial of 1954 before and after a booster inoculation 1 year later in 1955 and finally their antibody status in 1957. Thirteen of the children were negative to all 3 types before primary immunization in 1954. Their 1955 and 1957 titers are shown in Figure 77. Considering the fact that a 2 year period had elapsed since the booster inoculation the height of the present titers argues that immunity has endured.

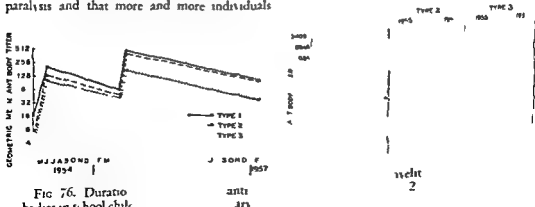


FIG 76. Duration of antibodies in school children and secondary inoculation (63 subjects—

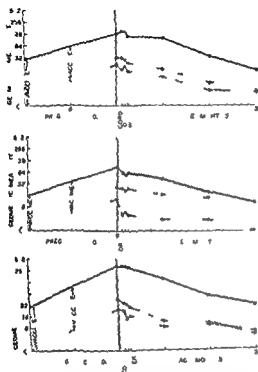


FIG 78 (Top) Type 1 serologic response of pregnant women to poliomyelitis vaccine and passive transfer of antibodies to infants

FIG 79 (Center) Type 2 serologic response of pregnant women to poliomyelitis vaccine and passive transfer of antibodies to infants

FIG 80 (Bottom) Type 3 serologic response of pregnant women to poliomyelitis vaccine and passive transfer of antibodies to infants

I would call attention to the data presented by Dr. Koprowski on persistence of antibodies in which some of his titers decreased perhaps two to fourfold. In a recent publication by Dr. Lenette the titers following actual paralytic infection in individuals decreased over 2 years approximately fourfold and the figures agree very closely with ours 7 years following the booster inoculation.

Figures 74 to 80 present another type of study which we have conducted on the duration of antibodies passively transferred to their infants by vaccinated and unvaccinated mothers. Fig-

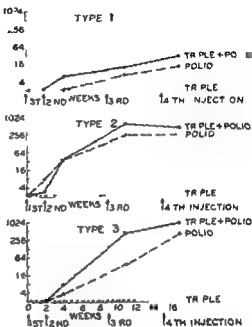


FIG 81 Geometric mean poliomyelitis antibody titers in monkeys 2 weeks after each of 4 vaccine injections

ure 78 shows the behavior of Type 1. Figure 79 of Type 2 and Figure 80 of Type 3 antibodies. It is of considerable interest that the rate of decline of all whether naturally or artificially evoked in the matter was approximately the same.

We are currently engaged in additional research which by its nature requires a killed virus vaccine that is immunization with multiple antigen preparations. We have obtained satisfactory responses in laboratory animals inoculated with a preparation containing diphtheria and tetanus toxoids, pertussis vaccine and trivalent poliomyelitis vaccine (Fig. 81). Certain problems of concentration, preparation and preservation remain to be solved as well as of dosage and administration. But the results so far have shown considerable promise. Perhaps in the near future manufacturers may combine these components and protect children against several diseases with one multivalent vaccine.

PROF. DR. DICK: There are 3 stages in testing live polio virus vaccines. The first consists of laboratory tests of the strains to be used.

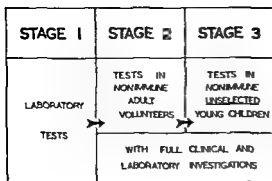


FIG 82 The 3 stages in the testing of live polio virus vaccines

and the second consists of tests of these strains in nonimmune adult volunteers. Dr Sabin's rigid laboratory tests of his attenuated viruses justified his proceeding to stage 2 the results of which he has presented to us. These first 2 stages are however a preparation for the third stage which consists of feeding the viruses to *unselected* nonimmune young children (Fig 82). This is the most important stage for we know from our studies of Dr Koprowski's Type 2 TN strain that there may be great differences in the multiplication of polio virus in the alimentary tract of children as compared with adults. My reason for saying *unselected* young children is as follows. If a group of institutional children devoid of antibodies is taken for testing oral vaccines these children may have already been naturally selected for a degree of resistance. Dr Koprowski's results with TN Type 2 vaccine which we could not confirm may have been due to the fact that he unwittingly selected children who were naturally poor hosts for polio viruses. In the third stage a qualitative and quantitative comparison between the virus excreted by vaccinated children and natural virus strains from paralytic and inapparent infections in children is required.

For various reasons the tests at stages 2 and 3 should be done at different times of the year for we cannot conclude that the same results will be obtained when live virus is fed in January as in September or at other times of the year in tropical countries.

It may be found that virus which is excreted after vaccination spreads from vaccinated individuals to their contacts. If spread does not occur then all that is required for safety is to

show that the virus which the children excrete after vaccination is less virulent than naturally occurring paralytic strains.

If on the other hand spread *does* occur and we found this with Dr Koprowski's Type 1 SM strain then it must be shown that either the excreted virus differs from naturally occurring strains and is more attenuated or if the excreted virus is as avirulent as naturally occurring strains then we must be assured that the natural virulent strains do not become virulent on passing through a community for if they do then the excreted vaccine viruses may do so too.

Finally what antibody level will be produced by avirulent virus vaccines which pass the tests of stage 3? Dr Sabin has not given us any antibody data from his studies in adults. It may be that the more highly attenuated strains will behave like the highly attenuated yellow fever or influenza viruses and fail to produce antibodies. Are we then between the devil and the deep blue sea—strains like SM Type 1 and TN Type 2 both of which after multiplication in the human gut become unacceptably virulent but are antigenic as against strains which may be acceptably avirulent but of poor antigenicity? The titers of antibody which we achieved with the partially attenuated SM and TN viruses were no better than those obtained after 2 injections of formalised vaccine.

Dr Sabin has suggested that the immunity resulting from ingestion of attenuated strains may be as long lasting as that following natural infections. I know of no evidence that durable immunity follows natural infection with non-invasive strains. Dr Koprowski's claim for the durability of antibody for 6 years refers to vaccination with a strain which produced virulent fecal virus after vaccination. If poor levels of antibody are produced and the immunity is not durable is this going to be improved with more highly attenuated strains? It may be that the best we can hope for is a *super* Salk vaccine using inactivated purified virus which might be expected to produce higher and more durable levels of antibody than the available formalised vaccine provides.

DR HORSTSMANN. The 3 papers have given a comprehensive picture of the problems which we now face in connection with the possible use of live attenuated polio viruses for vaccina-

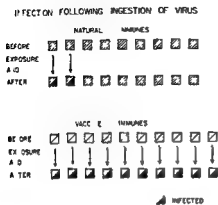


FIG 83 Responses of natural and vaccine immune persons to ingestion of  $10^{7.4}$  TCD<sub>50</sub> of LSc strain of attenuated Type 1 polio virus

tion. The work also brings out how much has been learned about human poliomyelitis as a result of investigations concerned with the enhancement of immunity. This is particularly apparent in connection with the nature of immune mechanisms in this infection.

Because they are easy to define and measure we have concentrated on circulating antibodies as indicators of resistance but more and more evidence seems to be accumulating that antibodies are not the whole story and that probably local immunity or so-called tissue immunity also may play a role. The data presented this morning by the 3 speakers all bear on this point. Unless the intestinal tract has participated in active infection with live multiplying virus it remains completely susceptible to viral implantation even in the presence of significant antibody induced by killed vaccine.

In a human trial of the LSc strain of attenuated Type 1 virus generously made available to us by Dr. Sabin we also have observed marked differences between responses of children with vaccine-induced antibodies as compared with children with naturally acquired antibodies. As shown in Figure 83 when fed a large dose of this strain of virus (10,000,000 tissue-culture infective doses) all 10 children with vaccine-acquired antibodies (100 per cent of those fed) readily became infected, excreted virus for a long time, developed neutralizing and complement-fixing antibodies. In contrast

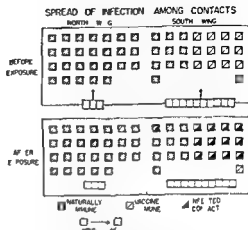


FIG 84 Spread of Type 1 LSc strain of attenuated polio virus among contacts of persons infected following ingestion of virus

only 2 of 9 naturally immune children became infected. These 2 excreted virus briefly, developed rises in neutralizing antibodies but no CF response.

We also measured the degree of spread which might occur when attenuated viruses are fed to children in a closed community. Our observations with the LSc strain and more recently with Dr. Sabin's KP 34 strain of Type 3 indicate that when these attenuated strains are fed to children in a group all of whom possess antibodies either naturally acquired or vaccine-induced the agents spread rapidly and readily to susceptible contacts. The speed of spread is indicated by the fact that contacts were already found to be excreting virus 4 to 6 days after the test subjects had been fed.

Figure 84 shows a comparison of the rates of spread among vaccine-immune and naturally immune contacts in this unit of 69 individuals. Essentially 13 infected persons who had been fed virus were introduced into the group. What happened was that 1 of 36 or 3 per cent of the naturally immune contacts became infected while for the vaccine-immunes the infection rate was 17 of 13 or 93 per cent, a figure remarkably similar to the infection rates reported by Dr. Fox and others for infection of fully susceptible children exposed to virus in their homes.

TYPE 1 ANTIBODY LEVEL PRE- EXPOSURE	VACCINE NUMBER	IMMUNE %	NATURALLY NUMBER	IMMUNE %
0-4	5/5	100	(1/1)	-
4-16	2/2	100	-	-
32-64	8/8	100	2/15	13
128-256	0/1	-	1/20	5
512-1024	-	-	0/9	0

FIG. 85 Relation between antibody titer at time of exposure and infection in 69 individuals. Asterisk indicates susceptible individual.

The role of the level of antibody in conditioning responses of the 2 groups vaccine and naturally immune is considered in Figure 85. In this Type 1 trial only in the range of 32 to 64 neutralizing antibody level were there sufficient persons in each group for an adequate comparison. Here 8 of the 8 vaccine immunes became infected while 2 of the 15 or 13 per cent of the naturally immune persons became infected. It has been suggested that higher levels of vaccine acquired and naturally acquired antibodies may be capable of preventing alimentary infection. At present however there is no indication that this is the case.

Taken together the data reported point to the relative nature of immune barriers in poliomyelitis and the dependence of this immunity on a balance or ratio between antibody level dosage of virus virulence of the infecting strain and probably previous experience or lack of it of the alimentary tract with the virus. That this naturally acquired immunity is more solid in terms of infection than that acquired from killed vaccine is indicated by the field experience and by the controlled human trials. However I agree with Dr. Sabin that the results thus far achieved with the killed vaccine in terms of the reduction of paralytic poliomyelitis demand its continued use in countries where economically feasible to see what it will do over the course of years. In the meantime however trials on an experimental basis calling for the cautious use of attenuated strains orally also seem warranted.

Because of the ease of spread of attenuated viruses the problem of the stability of these

strains becomes as important for the community as for the individual. Evidence has been presented today and our experience agrees with this that even the most highly attenuated strains in certain individuals may show some tendency toward increased neurotropism although by comparison with wild strains they remain still highly attenuated.

One argument is that it would seem desirable to replace the wild virulent strains which are so widespread in many areas at all times with highly attenuated strains. This argument has logic and force but the practical problem of how to go about doing this and how to evaluate the results adequately is for the proponents of this plan to provide. In highly endemic areas where the wild strains are most prevalent the incidence of paralytic poliomyelitis is very low and the disease is confined to the infantile age group. In this situation it would be necessary to feed attenuated viruses to enormous numbers of children over a long period of time to achieve statistically significant results. It would also be difficult to administer such a program successfully in underdeveloped areas requiring as it does careful follow-up and reporting of cases and extensive testing of excreted virus to find out if the attenuated strains are actually replacing the virulent ones and if so for how long.

Nevertheless in spite of the difficulties I for one hope to see such experiments carried out in the near future. Perhaps one way to minimize the difficulties would be to use the attenuated strains in areas where epidemics are making their appearance for the first time. If attenuated virus vaccine could be administered at the beginning of epidemics particularly those in relatively small population groups perhaps on islands an evaluation of the procedure could be achieved although only after repeated trials of this sort.

Another possibility would be a trial in a small country which has had and may expect to have epidemic poliomyelitis and has not yet used killed vaccine on any scale. There is much to be said for such a trial at this time using the most attenuated most stable and most antigenic strains. This is a bold program but no more so than was the field trial with the killed vaccine and now as then it would seem that the right mixture of boldness and caution are needed.

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PROF LÉVINE In countries where poliomyelitis is rare and where it remains strictly infantile the resistance normally acquired by the population comes at the same time from a spontaneous absence of early infection by the 3 types of the viruses and the prevalence of relatively attenuated strains which infect these populations in conditions which ensure a fairly equal distribution of the different antigenic types. Hence the temptation for the research worker to try to reproduce artificially what nature does spontaneously in countries where this natural immunization predominates this of course provided that the method chosen does not involve greater risks than those which result from spontaneous infection.

This obvious limitation led us to think in terms of using attenuated viruses no longer as a method of primary vaccination but as a means of enabling us to re-enforce possibly at a second stage and for prolonged duration the protection obtained earlier at a first stage through a vaccination by an inactivated virus.

Our tests have taken place on the chimpanzee exclusively. They were reported recently at the Conference on Cellular Biology Nucleic Acids and Viruses which was held in the Academy of Sciences of New York January 7 to 9 1957. If

these tests have indeed confirmed the theoretical possibility of obtaining such a re-enforcement of the basic immunity by the oral administration of the attenuated polio virus for the two following reasons our conclusions have stressed the express reservations concerning the practical possibilities of applying this.

1 The vaccinated subjects become virus carriers which does restrict the possible application of this method to communities or to groups where all the individuals have been given a prior basic immunity.

2 The strains of the polio virus which have been totally attenuated only yield an irregular and short-duration response with the vaccinated animals.

The findings of Dane Dick and their co-workers which have been published since dealing with vaccinated subjects in Northern Ireland with a modified attenuated virus have confirmed the first of these reservations by showing the true danger of relapse to the primitive virulence of the vaccine strain by the adaptation of the virus vaccine to the intestinal medium and its transformation into a wild strain.

The second reservation which is even more serious deserves some comment. Our monkeys indeed have been vaccinated and have received their basic immunity with a vaccine prepared on the basis of the method advocated by the Pasteur Institute. This is inactivated vaccine made of a mixture of the inactivated culture of the 3 types of virus with the following main characteristics: (1) the use of selected strains with a high antigenic power nonpathogenic by subcutaneous or intramuscular route (2) culture medium entirely synthetic and absorption of the culture on synthetic resin ensuring the absence of serum proteins or animal proteins from the finished vaccine (3) inactivation of the virus in 2 stages through an action of 2 antiseptics at low doses bringing about total inactivation in 77 hours and finishing the operation at a low temperature which guarantees the integral preservation of the antigenic power (4) no filtration of the cultures on ultrafine canulles which facilitates retention of all the virus without any loss of antigens (5) mixture of the monovalent lots in the final polyvalent vaccine not by volume but in terms of their antigenic potency for each of the lots.



Under such conditions such a vaccine used for a basic immunization with a dose of 3 subcutaneous injections 3 weeks apart determines with the animal as with man an immune response so strong that the peroral administration of attenuated strains of the polio virus is not followed by reaction and in fact there is no increase of the antibody titer.

If enough time is allowed to elapse say a year for the basic immunization due to the inactivated virus to begin to weaken and then we begin to administer through the mouth an attenuated virus we are confronted with the following alternatives which we have not been able to avoid so far.

Either the attenuated virus then administered has lost sufficient neuropathogenicity so that it may be considered as innocuous in which case it evoked a weak and irregular response the antibodies increasing in an inconstant manner and the titer remaining in any case lower than that which you would get through a simple booster injection of the inactivated vaccine or the virus then is still capable of provoking a response in the organism and evidently has adequate antigenic power. However in that case we always deal with strains which have not completely lost their neuropathogenic potency and therefore we cannot consider them as giving us the sufficient security guarantee for them to be applied without discrimination to man.

As it was impossible to avoid this alternative we gave up following this approach in our tests. Such test might well be taken up again with strains the certain innocuity of which would allow for their introduction in the organism by a parenteral route.

One should not forget that the digestive tract is after all an invaginated portion of the exterior medium and that it is not strictly speaking an integral part of the organism. It is host normally to a number of bacteria and viruses against which the organism reacts only if they show a certain aggressiveness.

If the attenuated viruses which we administer orally are sufficiently attenuated not to be aggressive any longer there is no valid reason to expect the organism to become immunized against them. If they preserve sufficient aggressiveness to provoke an antigenic response they keep in that way the very characteristics of a potentially dangerous virus.

That is why in spite of the positive value that we do recognize generally speaking in vaccination by means of attenuated viruses and in spite of the results already obtained in experiments in the present circumstances we must give some preference to vaccination by means of inactivated vaccines provided that vaccines giving necessary guarantees of security and antigenicity such as those we have been using. They alone will give us the results that we think we can achieve.

# Immunization Against Poliomyelitis in the Light of Existing Immunity of Populations

DR A W W PAYNE

I have tried to present the information that I have been able to collect regarding the status of immunity of populations measured by different methods in different parts of the world known to health authorities in deciding whether or not to apply vaccination and if so to which age groups it should be applied.

A decision to introduce immunization against a communicable disease depends on the danger of exposure to the disease and the presence of a susceptible population. The danger of exposure to poliomyelitis obviously depends in the first place on the presence of the 3 types of poliovirus. The result of serologic surveys and virus isolation has shown that all 3 types of poliovirus are present more or less continuously in all populations except in relatively small and isolated communities where the infection is reintroduced at intervals which may be quite lengthy in which event the well known virgin epidemic occurs. The apparent absence of poliomyelitis in a community may be due to two totally different reasons. On the one hand the infection may be so highly endemic that infection occurs at a very early age and causes very few or no neurological symptoms. As is well known a second reason occurs in the tropics. On the other hand the absence of cases may be due to the absence of virus as has been demonstrated in the first case. In the former case immunization is not necessary whereas the latter population is in a most precarious position because the introduction of a virulent strain could followed by a disastrous epidemic.

These are the two extremes but closely analogous situations exist in different sections of large non-tropical populations and even perhaps in the same town. In this is of considerable importance to those who are planning polio vaccine control at the national level.

Assuming the presence of the 3 types of poliovirus in a country susceptible to reflect in the incidence and the age incidence of paralytic disease. I would emphasize the importance of studying paralytic cases rather than all cases. The clinical diagnosis of paralytic poliomyelitis is not a specific diagnosis since many other conditions can be mistaken for it. Two thirds are not due to poliovirus and only one third on these cases might have a large influence on the results.

When examining the susceptibility of populations by the study of the incidence of the disease, greatest stress should be laid on the incidence since the actual incidence depends on other factors in addition to susceptibility and as year figure changes greatly from year to year. The figures found in different parts of the world attack rates expressed as percentages of the maximum age specific attack rate and they are plotted on a logarithmic scale.

In Sweden the attack rate of over 75 years of age is still nearly 50 per cent of the maximum rate which occurs in the age groups 1 to 4 and 5 to 9 whereas in Israel the attack rate at the age of 5 is one tenth of the maximum attack rate in the first year of life is only 15 per cent of the maximum whereas in Israel it is 80 per cent of the maximum rate. However there are special circumstances in Israel which may account for this. The other examples shown the intermediate positions.

Figure 8C also shows the differences in age distribution of cases found within a country in the United States the relative incidence in the 5 to 9 and older age groups in the so-called epidemic to be less than in the rest of the countries. In Sweden

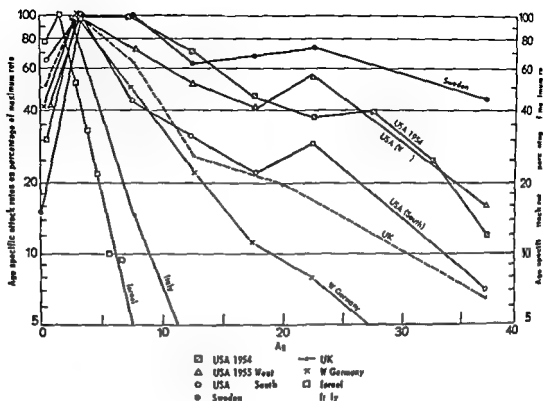


FIG 86 Age incidence in some countries

land a similar difference is to be found between different cantons for example in Lucerne and Berne there is a higher incidence in older age groups than in Geneva and Fribourg. In some countries a similar difference has been shown between urban and rural areas the latter generally having a higher incidence in the older age groups.

I have no satisfactory figures to show the different age distribution of cases in different population groups in the same town but the observations of Walton and Melnick suggest that it may be considerable the highest socio-economic groups showing a pattern similar to that of Sweden and the lowest approaching that of Egypt. The results of serologic surveys support this view. The importance of this in designing vaccination programs is obvious. The differences in different parts of a country may sometimes be great enough to justify the complication of immunizing different age groups and in some countries consideration certainly should be given to immunizing older age groups in upper-socioeconomic groups.

Obviously in addition to age incidence the mean incidence over a period of years must be a major factor in influencing a decision as to whether or not to introduce immunization. In countries with good reporting systems this presents no difficulties although it may be well to stress again that only paralytic cases should be considered. In other countries reported figures may be quite meaningless. A good example of this was Egypt. Between 1944 and 1951 the mean annual number of cases reported was 9.5. In the course of their classic studies in Cairo, Paul and his colleagues estimated that the real attack rate in that city lay in the neighborhood of 4 to 8 per 100 000 population. The correctness of this estimate has been shown by subsequent events.

You heard yesterday the official delegate from Egypt estimate the attack rate as about 9 per 100 000 population. According to the official figures received by WHO the rate is about 5.2 per 100 000 population practically all of which are paralytic. But I think it is safe to assume that official reporting is still incomplete and

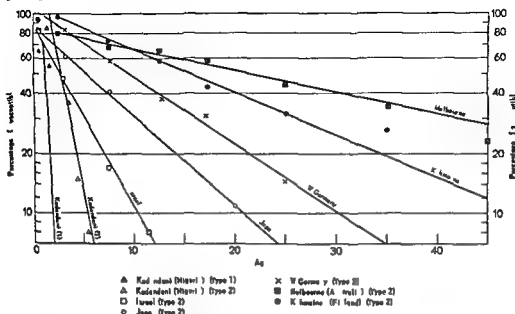


FIG. 87. Percentage of susceptibles to Type 2 virus in some areas (Linear scale of age)

cially in rural areas. The median incidence of polio in the United States in recent years has been of the order of 20 per 100 000 of which between one third and one half were nonparalytic giving an incidence of about 10 to 15 paralytic cases per 100 000 population. Therefore it may be said that the problem of poliomyelitis in Egypt is not really much less than it is in the United States a very different assessment to the assessment generally made. This is doubtless because in Egypt other health problems overshadow poliomyelitis as a cause of disease.

Statistics regarding the incidence of poliomyelitis must be interpreted differently from those for many other communicable diseases where the outcome in recovery and sequelae are rare or unimportant. In poliomyelitis anything more than minimal paralysis is permanent so that the morbidity is in effect cumulative.

A recent study by Payzin in Turkey suggests that what has been found in Egypt may be true of other countries in that part of the world. The suggestion here is of course that there is a large number of undetected cases in many countries and now that we have an effective means of exercising a large measure of control

over paralytic disease the problem of poliomyelitis must be reassessed.

How is this to be done? Although a large measure of success was obtained in Egypt by efforts to improve reporting there are many areas in the world where this is quite impracticable at present. It would seem however that valuable information might be gained by properly designed clinical surveys such as those carried out by Paul in Cairo looking for a residual paralysis. The second method of estimating susceptibility and assessing the poliomyelitis situation is by means of serologic surveys for neutralizing antibodies which provide a record of the past experience of the population.

Thanks to the generous co-operation of members of the WHO Expert Advisory Panel on Virus Diseases and of many other virologists many of whom are present today I have been able to collect about 100 surveys in more than 40 different countries. The presentation and the interpretation of such a mass of data from such diverse sources is a matter of considerable difficulty. Different methods of selecting the study population, different laboratory techniques and different serum dilutions have been used. Some surveys are quite inadequate in size whereas a

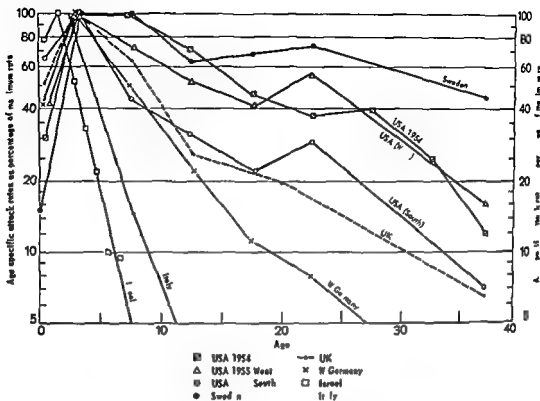


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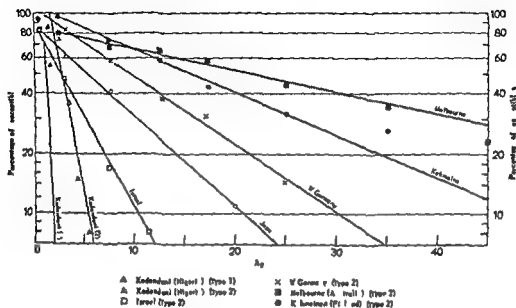


FIG. 87. Percentage of susceptibles to Type 2 virus in some areas (Linear scale of age)

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few are very large indeed. In addition there is the well known variation in the results of a laboratory test both between laboratories and within laboratories on repetition of the test. To illustrate I will mention the work of Olin and Wesslen who found that on retesting 200 sera 141 per cent gave a different result. To this must be added sampling and other errors mentioned above so that it is evident that most surveys can be regarded as showing only a rough approximation to the real situation. In view of this I have adopted a method of approximation which seems to have some advantages.

In Figures 87 and 88 the vertical scale shows the percentage of susceptibles remaining at a given age plotted on a logarithmic scale. The horizontal scale is the age generally on a linear scale though there is sometimes an advantage in using a logarithmic scale for age as well. Below 10 per cent the errors in these surveys and the small figures make divergence from the straight lines rather unimportant—rather without much meaning. When many surveys are plotted in this way they fall rather closely along straight lines. As you will see the fit is quite good.

Figure 87 shows susceptibles to Type 2 virus in Melbourne in Kuhmoinen in Finland West

ern Germany Japan Israel and Kadandani which is a village in Nigeria. These I have selected as being representative of the various patterns of the serologic survey scene. Fifty per cent of these populations are susceptible at the age of 22 in Melbourne 15 in Kuhmoinen 9 in Germany 5 in Japan and 2 in Israel and Kadandani. The 10 per cent level is not reached at all in Melbourne and Kuhmoinen. It is reached at the age of 30 in Germany 21 in Japan 10 in Israel and 5 in Kadandani. Below the 10 per cent level as I have already said the small numbers make the changes in percentages of doubtful significance. But it does seem that in most surveys the complete absence of susceptibles is unusual and there is often a tendency for the percentage to show an increase in adult life. Whether this is due to loss of antibody below the level of sensitivity of the test or to some other cause is a matter for speculation. These 6 surveys show the most usual patterns in areas of different endemicity. All intermediate patterns may be found. The most rapid loss of susceptibility that I have been able to find was to Type 1 virus in Kadandani. One hundred per cent of 2-year-olds were already immune. This presumably was due to a recent epidemic wave of infection but apparently there were no cases of paralysis. However even

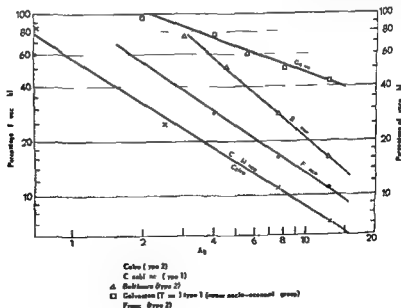


FIG. 88. Percentage of susceptibles (Logarithmic scale of age)

TABLE 64 SURVEY OF GROUP 1

PROTOTYPE ISRAEL  
50% < 5      10% < 10

Belfast	Chile Type 2
Londonderry	Cuba Havana Type 2
Glasgow (lower)	Eritrea
Glasgow (upper) Type 1	Sudan
Italy (Palermo)	Egypt Cairo Type 2
Italy (Naples)	French Morocco Casablanca
Italy (Milan Brescia, Novara)	Nigeria Kadandani
U.S. Galveston (lower)	Madagascar
U.S. Texas (lower) Type 2	Mauritius
U.S. Phoenix (lower)	Liberia
U.S. Los Angeles (lower) Type 2	Belgian Congo
Canada St. Augustin Type 2	India Bombay
Canada Roberval Type 2	Turkey
Uruguay Canelones	Aden Type 2
Venezuela Caracas	Tahiti
Brazil Rio de Janeiro (lower)	Raiatea

TABLE 65 SURVEY OF GROUPS 2 AND 3

GROUP 2 PROTOTYPE JAPAN  
50% 3-6      10% 10-20

N. Ireland (counties)	U.S. Baltimore Type 2
England (1924)	U.S. Louisiana
Liverpool	U.S. Charleston (lower)
Bethesda	U.S. Cincinnati Type 2
France (provinces)	U.S. Thomasville
France (Paris Assistance publique)	Canada Montreal
Belgium (Brussels)	Uruguay Montevideo
Belgium (Flanders)	Korea Type 2
Switzerland (Geneva)	Okinawa Type 2
Serbia Types 2 and 3	N. Australia (aborigines)

GROUP 3 PROTOTYPE W. GERMANY  
50% 6-10      10% 20-30

Freiburg	Iceland Type 2
Marburg	Rumania Bucharest Type 2
Munich	Hungary Szeged Type 2
Italy (Genoa)	U.S. Galveston (upper)
Sweden (Norrbottens län)	U.S. Miami Type 2
Finland (Helsinki)	Brazil Rio de Janeiro (upper)
Finland (Jorvasku)	Hong Kong
Finland (Helsinki)	Singapore

in this highly endemic area a few individuals over the age of 40 showed no antibody to 1 or 2 types although in no instance was there no antibody to all 3 types over the age of 1 year.

On the basis of this figure it is possible arbitrarily to divide the majority of surveys into 5 groups.

Group 1 those in which the percentage of susceptibles diminish as rapidly or more rapidly than in Israel.

Group 2 those lying between the graphs for Israel and Japan.

Group 3 those between Japan and Western Germany.



TABLE 66 SURVEY OF GROUPS 4 AND 5

GROUP 4 PROTOTYPE KUHMÖINEN  
50% 10-15      10% 10-50

Glasgow (upper) Types 2 and 3  
Hamburg  
Switzerland (Vaud)  
Sweden Stockholm Type 1  
Sweden Got o Bohus Län Type 1  
Sweden Göteborg  
Sweden Kronoberg

Finland Åland Is  
U S Allegheny  
U S Winston Salem  
U S Phoenix (upper)  
S Australia  
W Australia Como  
New Zealand Otago

GROUP 5 PROTOTYPE MELBOURNE  
50% >15      10% >50

Sweden Stockholm Types 2 and 3  
Sweden Got o Bohus Län Types 2 and 3  
U S Charleston (upper)

Canada Eskimos  
Alaska Eskimos  
Serbia Type 1

Group 4 those between Western Germany and Kuhmöinen

Group 5 those acquiring antibody more slowly than in Kuhmöinen

In placing surveys into various groups great est emphasis has been placed on the age at which 50 per cent susceptibles remain. In some surveys only that figure was available since a wide-enough age group was not examined. In others the 10 per cent level was approached early but not reached.

In most surveys of adequate size the graphs for all 3 antibody levels fall into the same group. In a few instances where the differences are very marked the types are specified. Where only Type 2 antibodies have been tested this is also specified. In deciding whether to place a survey in one of two neighboring groups if it is a borderline case preference is given to the findings for the Type 2 antibody. However it should be remembered that this method of presentation is a rough approximation only. It is quite impossible to present all these results in full detail and the inherent errors of these surveys must constantly be borne in mind.

In Table 64 the surveys of the first group are shown. I would like to point out that the upper socioeconomic division in Glasgow falls into this very early acquisition of antibody with respect to Type 2. Types 2 and 3 for Glasgow we will find in Group 2 which we come to later. The patterns found in the lower socioeconomic groups of highly developed countries are quite

comparable with those found in the most backward countries. Table 65 shows Groups 2 and 3. Here you will find that for Serbia Types 2 and 3 fall into Group 2 but later in Group 5 you will find Serbia Type 1 which is very very strange. Hong Kong and Singapore both rather surprisingly fall into Group 3 although in both instances it was the lower socioeconomic groups which were examined. In Table 66 are Group 4 and Group 5. Much as you would expect countries with a severe polio problem fall into this Group 5.

Where the necessary information was available I have also studied the percentage of susceptibles remaining at the age below which 90 per cent of all cases have occurred or at which the attack rate has fallen to one tenth of the maximum rate. There is some variation but in the majority of instances between 20 and 30 per cent of susceptibles remain to each virus type at that age. In areas where the antibody pattern is that of Group 1 fewer susceptibles remain than in areas where antibodies are acquired more slowly. But evidently few cases are to be expected in the population with less than 20 per cent susceptibles.

Agreement between susceptibility estimated on the basis of age incidence and by serology is reasonably close. Very close agreement is not to be expected because in most instances the two populations are not identical, one perhaps being a small sample of a particular town the

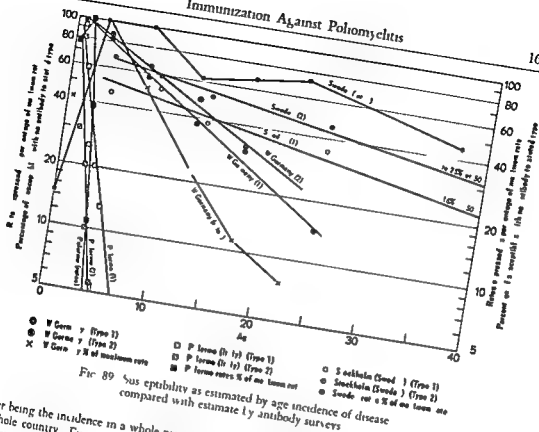


Fig. 89 Susceptibility as estimated by age incidence of disease compared with estimate by antibody surveys

other being the incidence in a whole province or a whole country. Figure 80 gives 3 representative examples. There is an error in this figure many have been interchanged. You will note that the acquisition of antibody has roughly the same slope as the decrease in incidence of cases in the cases decreasing a little more rapidly in each instance than the rate of acquisition of antibody. I am afraid that it time does not permit any more expansion of that subject.

The interpretation of the significance of the antibody in protecting in this disease is dependent on whether there is cross protection between the different types of virus. It has long been held that previous infection with Type 1 virus gave some cross immunity to the other types. That was discussed by Lepine at the last Conference but since then review earlier evidence further but since then as Salin has shown the monkeys challenged with Type 1 virus less frequently develop paralysis if they already had Type 2 antibody produced by vaccination or by infection. Salin

yesterday read to us some of his work on the interrelationship of the Types 1 and 2 and 3 having antigenicity in common.

Material which was kindly sent to me by Brown supports this view. In the 1954 trial an extensive survey showed that in an area 50 per cent of the study population had antibodies to Type 1 before vaccination. A study of antibodies in cases occurring during the trial confirmed by virus isolation showed that out of 17 paralytic cases from which Type 1 virus was isolated only 7 had antibody to Type 1 virus. There was no relationship between the presence of Type 2 antibody and paralysis due to Type 1 virus. One would have expected that 50 per cent of the cases would have had Type 2 antibody. Thus there are 74 cases with Type 2 antibody missing from the paralytic group.

Figure 90 shows the results of serologic surveys in different socioeconomic groups in Charleston W. Va. The straight lines approximate the rates of development of Type 2 antibody in the 2 socioeconomic groups upper and lower. The lower group I would point out up

# POLOMYELITIS INDIA

1952-1953

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TOWN

TYPE	NO.	PERCENT
TYPE 1	10	10
TYPE 2	20	20
TYPE 3	30	30
TYPE 4	40	40
TYPE 5	50	50
TYPE 6	60	60
TYPE 7	70	70
TYPE 8	80	80
TYPE 9	90	90
TYPE 10	100	100

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quite impossible to pr  
full detail and the in  
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In Table 64 the surveys  
shown I would like to poin  
socioeconomic division in GI  
very early acquisition of anti  
to Type 2 Types 2 and 3 for  
find in Group 2 which we con  
patterns found in the lower  
groups of highly developed count

Type 2 antibodies but they are not enough. Consequently there are considerable contingents of populations in our country who need protection against poliomyelitis and we are going to give them this particular protection by means of vaccination with the vaccine produced in our country.

NOTE: Dr Chumakoti's focus is in Rangoon. He applied the enterovirus with an Enrichment in which this strain was taken. Unfortunately any additions that may have been made could not be listed.

DR GHARAPLE: I present a few facts which will explain existing conditions on the basis of which I have been carrying out my work. Figure 91 is a map of India and I have depicted by triangles the areas where paralytic poliomyelitis exists. The triangles with the darkened upper corner show areas where it is notifiable. Thus it will be seen that out of a large country the disease is notifiable in only

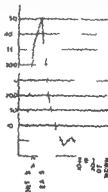
one third of it. The notifiable paralytic cases generally have been about 1000 each year. I roughly calculate that the incidence would be about 10 per 100,000. In the child population it may be about 4 or 5 times as high.

There are ups and downs during these 11 years which are more or less constant for the last 8 years. Figure 92 shows the age incidence. These figures are from Bombay but they more or less apply to the rest of the country as well. Here you will find that it is restricted to the child population, the peak being at 1 to 2 years.

Figure 93 shows the seasonal incidence. It is concentrated in a few months, May to September. When the figures over a number of years are plotted along with the rainfall, one sees some rather interesting results (Fig. 94).

There are ups and downs during these 11 years and the peaks to some extent correspond to high rainfalls. We have never been able to ex-

	1948	1949	1950	52	1953	1954	1955	1956	TOTAL
0-6 MONTHS	5	5	0		3	8	10	5	
6-12	83	5	0	5	23	0	25	5	318
12-18	6	40	1	55	38	10	5	9	49
18-24	5	10	27	5	2	3	16	23	
24-30	8	0	0	0	4		9	0	0
30-36	5	5	5	2			0	23	
36-42	5	0	0	5	5	5	0	41	
42-48	5	0	0	0	0	5	0		
48-54	5	0	0	0	0	0	0	1	
54-60	0	0	0	0	0	0	0	0	2
60-66	0	0	0	0	0	0	0	0	
66-72	0	0	0	0	0	0	0	0	
72-78	0	0	0	0	0	0	0	0	
78-84	0	0	0	0	0	0	0	0	
84-90	0	0	0	0	0	0	0	0	
90-96	0	0	0	0	0	0	0	0	
96-102	0	0	0	0	0	0	0	0	
102-108	0	0	0	0	0	0	0	0	
108-114	0	0	0	0	0	0	0	0	
114-120	0	0	0	0	0	0	0	0	
120-126	0	0	0	0	0	0	0	0	
126-132	0	0	0	0	0	0	0	0	
132-138	0	0	0	0	0	0	0	0	
138-144	0	0	0	0	0	0	0	0	
144-150	0	0	0	0	0	0	0	0	
150-156	0	0	0	0	0	0	0	0	
156-162	0	0	0	0	0	0	0	0	
162-168	0	0	0	0	0	0	0	0	
168-174	0	0	0	0	0	0	0	0	
174-180	0	0	0	0	0	0	0	0	
180-186	0	0	0	0	0	0	0	0	
186-192	0	0	0	0	0	0	0	0	
192-198	0	0	0	0	0	0	0	0	
198-204	0	0	0	0	0	0	0	0	
204-210	0	0	0	0	0	0	0	0	
210-216	0	0	0	0	0	0	0	0	
216-222	0	0	0	0	0	0	0	0	
222-228	0	0	0	0	0	0	0	0	
228-234	0	0	0	0	0	0	0	0	
234-240	0	0	0	0	0	0	0	0	
240-246	0	0	0	0	0	0	0	0	
246-252	0	0	0	0	0	0	0	0	
252-258	0	0	0	0	0	0	0	0	
258-264	0	0	0	0	0	0	0	0	
264-270	0	0	0	0	0	0	0	0	
270-276	0	0	0	0	0	0	0	0	
276-282	0	0	0	0	0	0	0	0	
282-288	0	0	0	0	0	0	0	0	
288-294	0	0	0	0	0	0	0	0	
294-300	0	0	0	0	0	0	0	0	



	1948	1949	1950	1951	1952	1953	1954	1955	1956	TOTAL
JAN	0	5	3	5	9	4	0	3	0	27
FEB	0	0	0	0	0	0	0	0	0	0
MAR	0	0	0	0	0	0	0	0	0	0
APR	0	0	0	0	0	0	0	0	0	0
MAY	0	0	0	0	0	0	0	0	0	0
JUN	0	0	0	0	0	0	0	0	0	0
JUL	0	0	0	0	0	0	0	0	0	0
AUG	0	0	0	0	0	0	0	0	0	0
SEP	0	0	0	0	0	0	0	0	0	0
OCT	0	0	0	0	0	0	0	0	0	0
NOV	0	0	0	0	0	0	0	0	0	0
DEC	0	0	0	0	0	0	0	0	0	0
TOTAL	0	0	0	0	0	0	0	0	0	0

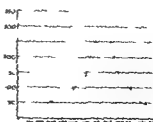


FIG. 92 (Top) Age incidence of poliomyelitis in India

FIG. 93 (Bottom) Seasonal incidence of poliomyelitis in India

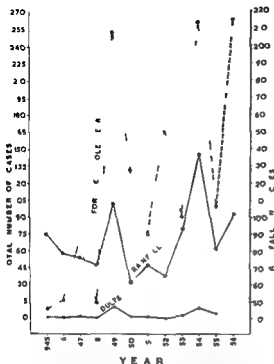


FIG 94 Number of cases of poliomyelitis compared with incidence of rainfall (Bombay 1945-1956)

plain exactly what this means. On an all India basis I have been able to collect some sera through the Armed Forces Medical College which is a training center where young men from 18 to 20 attend. Two hundred fifty nine sera have been tested so far and they show antibodies against all 3 virus types.

More particular studies were carried out in Bombay. Figure 95 shows quite a complex set of data. I will not refer to all of it. You will find that paralytic cases are scattered all over. Each year in the same regions cases recur and over 200 cases have been occurring from 1949 onward. In a total of some 1400 cases so far only 47 have been in persons above 14. Last year a small group of 3000 children were investigated. One hundred twenty four sera were studied (Fig 96) which showed that about 25 persons up to the age of 12 are susceptible. One hundred fifty two polio viruses have been isolated of these 117 were Type 1, 13 were Type 2 and 22 were Type 3. It was rather remarkable

that in the first 6 years Type 1 was the most common. Type 2 came up occasionally but, in 1955-1956 Type 3 paralytic poliomyelitis has occurred more frequently and on the borderlines of areas which previously were affected.

I would refer to another study carried out in the town of Dohad (Fig 97) 300 miles from Bombay where in 1957 an epidemic of poliomyelitis occurred with 112 cases in children in a population of 25000. Subsequently there have been 10 to 12 cases each year but poliomyelitis persists. Last year we started a house to house survey; the data are not yet complete but the sera study showed the same results, namely that up to the age of 10 or 12 25 per cent of the children are susceptible (Fig 98). Here again I might stress that poliomyelitis continues to exist.

The Andaman and the Nicobar Islands experienced a poliomyelitis epidemic in 1949, a report of which came up before the Second International Congress. Then for years nothing was heard. Suddenly in December 1956 I received a telegram from the Government of India saying that poliomyelitis had broken out on an adjacent island. Dr. Seal went up to the spot and I procured the materials for virus isolation and serology—more than 100 sera which show a good acquaintance or experience with all the 3 types of the virus. I will not analyze the data completely but I cite this as an instance that after a gap of several years poliomyelitis had broken out again.

I have mentioned these facts to explain the various types of existing conditions. In Bombay the same number of cases continues. In Dohad after a large sized epidemic a small number of cases continues. In Andaman a Bombay type epidemic continues and I begin to feel that unless measures of active immunization are undertaken poliomyelitis will not be controlled. Today in India vaccine is not manufactured and it is not being used on a large scale but on a voluntary basis for whoever wants it. The public and the doctors are vaccine minded and I am glad about it.

PROF. GIOVANARDI: Before expressing some observations on the application of vaccination against poliomyelitis in Italy I shall refer briefly

to the behavior of neutralizing antibodies as an index of the condition of natural immunity of a population

This study was performed on groups of population belonging to middle or low hygienic sanitary and socioeconomic classes of north and south-central Italy—Biella Ircinia Milan Novara Naples Bari Cosenza and Taranto The

results are summarized in Figures 99 100 and 101 As can be seen the neutralizing antibodies of the 3 types of polio virus occur with great frequency at birth decline progressively to a minimum at the end of the first year of life and then increase till reaching a maximum at the age of 5 to 6 years At such age in all groups of population the number of subjects with anti

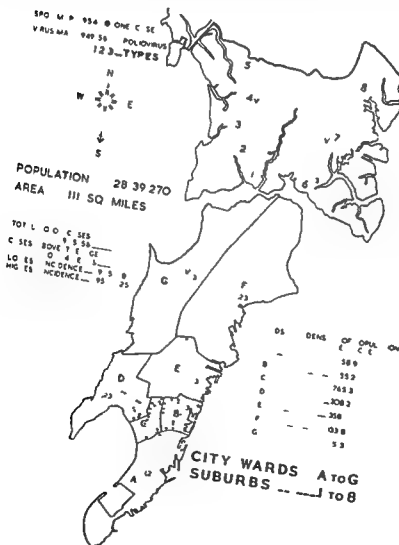


FIG 95 In iden e of pchomelatus in greater Bombay

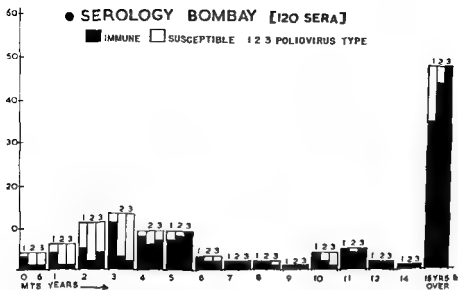


FIG 96 (*Top*) Results of serology tests in Bombay

FIG 97 (Left) Map of Dohad and its adjoining villages

(Right) Map of Dohad showing its wards

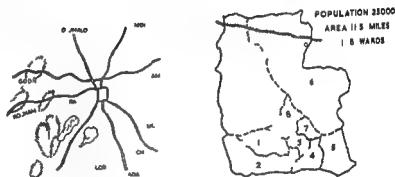
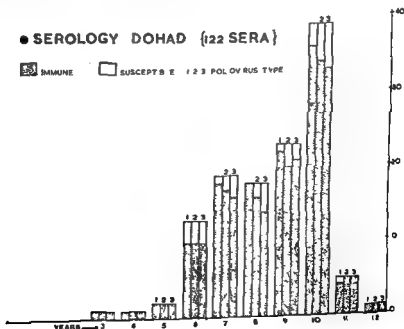


FIG. 98 (*Bottom*) Results of serology tests in Dohad



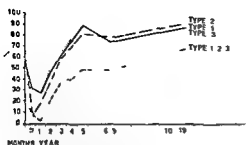


FIG 99 Behavior of the neutralizing antibodies against the 3 polio virus types in a group of subjects in Milan, Brescia and Novara in relation to age

bodies for the 3 types of polio virus is from 50 to 80 per cent and the number of subjects with antibodies for Type 1, Type 2 or Type 3 may reach 90 per cent and more.

According to official data about two thirds of paralytic poliomyelitis cases in Milan and Brescia and in all of Italy occur in the first 3 years of age.

The behavior of the complement fixation antibodies and for comparison of the neutralizing antibodies in 700 children of Naples and Cosenza is shown in Figures 100 and 103.

Under 7 years of age only a few children—5 to 10 per cent—have complement fixation antibodies. The number of children with these

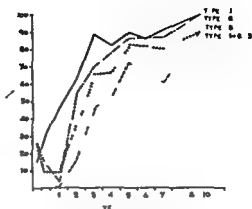


FIG 101 Behavior of the neutralizing antibodies against the 3 polio virus types in a group of subjects in Naples, Taranto, Bari and Cosenza in relation to age

antibodies then increases rapidly reaching rather high values about 50 per cent at 5 years.

In conclusion I would call attention to the following points:

1. In Italy the age range of those least protected by natural immunity is rather short and probably extends from the first year of life to the fourth and fifth year.

2. It follows that the application of vaccination is to be considered mainly for the children of the early ages up to the fifth year.

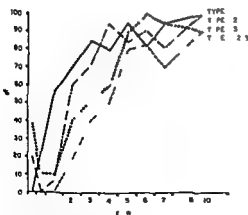


FIG 100 Behavior of the neutralizing antibodies against the 3 polio virus types in a group of subjects in Naples in relation to age

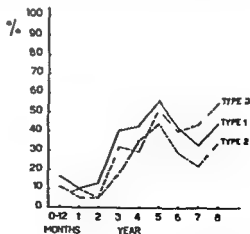


FIG 103 Behavior of complement fixation antibodies against the 3 polio virus types in a group of subjects in south Italy in relation to age



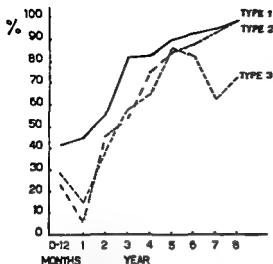


FIG 103 Behavior of the neutralizing antibodies against the 3 polio virus types in a group of subjects in south Italy in relation to age

3 In classes of population more refined from hygienic and economic standpoints or in isolated population groups in south Italy it is possible that the age period not protected by the natural immunity is wider and therefore that in these conditions vaccination deserves a more extensive application

4 The behavior of complement fixation tends to show that in Italy infection and reinfection from polio virus occur with a certain frequency at least till the age of 8. This is probably a favorable condition in which to employ vaccination with killed virus because the immunity roused by primary vaccination can be strengthened by these infections. This is not only for the amount of antibodies but also for the quality because it must be established whether the antibodies from killed virus from all protection standpoints are of the same potency as antibodies from living virus—from virus which multiplies in the human body

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DR GOLDSTEIN. I shall restrict my comments to three major points: the antigenic crossing between the polio virus types; the immunologic response of infants to vaccination with a killed vaccine; and the influence of mass immunization on the future relationship of polio virus to man.

The antigenic crossing among the 3 polio virus serotypes and especially between Types 1 and 2 advanced by Sabin and others and recently re-emphasized by Salk may be of greater importance in the epidemiology of poliomyelitis than has been previously anticipated and may have a bearing on mass immunization programs. Most of our information on the distribution of immunity to poliomyelitis has been gathered from surveys of Type 2 antibodies which undoubtedly is due to the fact that techniques for measuring these antibodies have been available for over a decade. Since practical methods for measuring Type 1 and 3 antibodies have become available only recently, not enough information is as yet on hand. Fortunately, as pointed out by Dr Payne, the occurrence of Type 2 antibodies is a fairly good index of the susceptibility of populations to paralytic poliomyelitis.

Table 67 is from a survey of polio antibodies carried out recently in our country illustrating this point. Antibodies to Type 1 polio virus develop very early and by the age of 18 months two thirds of the infant population have had experience with this type of virus. The acquisition of Type 2 antibodies comes later and lags behind Type 1 by almost 1 year. Thus by the age of 18 months only 19 per cent have Type 2 antibodies and two thirds is reached only 12 months later. In Israel Type 1 virus is responsible for over 80 per cent of paralytic poliomyelitis and all this occurs in infants and young children from 6 to 36 months of age.

Therefore it seems to me that the prevalence of Type 2 antibodies may be not only an important index for measuring the susceptibility of a population to poliomyelitis but also that its

## DISCUSSION

TABLE 67 RESULTS OF ANTIBODY TESTS OF ISRAELI INFANTS AND CHILDREN TO THE 3 POLIO VIRUS TYPES PREVACCINATION BLOODS

AGE GROUP (MONTHS)	NO TESTED	ANTIBODIES TO				WITHOUT ANTIBODIES TO ANY TYPE		ANTI-BODIES TO ALL 3 TYPES	
		TYPE 1	%	TYPE 2	%	TYPE 3	%	%	%
3-6	85	15	18	16	19	19	14	53	61
7-12	90	33	36	9	10	23	25	47	47
13-18	84	56	66	16	19	29	37	18	21
19-24	III	53	77	28	41	38	56	6	9
25-30	65	56	86	44	67	57	80	2	3
31-36	57	46	88	41	78	47	90	0	34
37-48	37	35	95	33	89	37	86	1	33

endemism may be of primary importance in determining the epidemicity of Type 1 virus and the resulting occurrence of epidemics of paralytic polio.

A situation different from that in Israel prevails in other countries like Nigeria, Morocco and other subtropical areas where Type 2 polio virus is highly endemic and at the age of 1 year over 50 per cent have already been infected with this virus.

Basic immunization with killed virus vaccine especially in infants and young children results primarily in the development of high titer Type 2 antibodies. Thus on mass vaccination an infant population is quickly transformed from a Type 2 nonimmune to a Type 1 immune population and this by itself without considering the Type 1 response may be of importance in preventing Type 1 epidemics.

This is now the situation in Israel. Serologic studies indicate the development of Type 2 antibodies to a high degree and rather low for Type 1. Nevertheless there is little paralytic polio this year as compared with previous years.

Although the immunologic response of infants to vaccination with a killed virus vaccine may not be satisfactory we have to face it in all countries where polio is a infantile type. Only future experience will answer the numerous problems involved in the vaccination of this low age group, problems such as the exact age at which to immunize, what amounts of vaccine should be administered, what schedule used and

so on. Without having these answers on hand we should proceed to immunize infants shortly after birth, say at the age of 3 to 4 months, since thus we will be able to prevent a substantial number of cases of paralytic poliomyelitis.

In what measure will mass immunization of a small part of the population—that of infants and very young children—influence the circulation of the different polio virus serotypes? We know and have been reassured this morning that intestinal infection may proceed almost undisturbed in a naturally immune or a partially vaccinated population. However will this continue for years to come or will the picture ultimately change and shall we create a polio virus free population? This is by far one of the most important problems which those responsible for public health will have to face in the future.

Dr. MOSKOWITZ: To give the right judgment on the immunity situation of the Italian population on an eventual application of a vaccination against poliomyelitis, researchers have been made on the distribution of the 3 polio virus types and on the incidence of neutralizing antibodies in different age groups.

I shall report on the etiologic results while those of the serologic researchers will be reported by Dr. G. S. Anand.

The distribution of the 3 polio types has been studied in different epidemiologic conditions on paralytic polio patients.

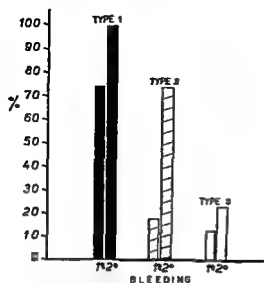


FIG 104 Behavior of the neutralizing antibodies in contacts (epidemic outbreak in Brescia 1953)

tients on population groups (noncontacts) in absence of paralytic polio cases in interepidemic periods on epidemic community outbreaks (contacts)

The results are summarized in Tables 68 to 71 and Figure 104

From our researches on the diffusion of the 3 polio virus types in the Italian population one can state the following

1 Most of the paralytic polio cases are caused by Type 1 virus frequently cases are caused by Type 2 and rarest are the infections caused by Type 3

TABLE 68 POLIO VIRUS TYPES ISOLATED FROM PARALYTIC POLIO PATIENTS IN DIFFERENT ITALIAN DISTRICTS FROM 1953 TO 1957

DISTRICTS	VIRUS TYPE			TOTAL
	1	2	3	
Lombardy	97	32	7	136
Piedmont	17	2	0	19
Trieste	2	0	0	2
Tuscany	0	3	0	3
Umbria	2	0	0	2
Campania	37	12	7	56
Basilicata	1	0	0	1
Apulia	5	0	0	5
Calabria	0	0	1	1
Total	161	49	15	225

TABLE 69 FREQUENCY OF POLIO VIRUS TYPES IN CASES OF PARALYTIC POLIO IN MILAN BRESCIA AND NOVARA

YEAR	STRAINS OF TYPE ISOLATED			TOTAL
	1	2	3	
1953	23	10	1	34
1954	30	0	0	30
1955	23	19	2	44
1956	37	5	4	46
Total	113	34	7	154

TABLE 70 POLIO VIRUS AND OTHER CYTOPATHOGENIC AGENTS ISOLATED FROM THE STOOLS OF 900 HEALTHY CHILDREN (NONCONTACTS)

POLIO		COXSACKIE		ECHO		ADENOVIRUS		CYTOPATHOGENIC UNIDENTIFIED AGENTS
STRAINS NO	TYPE	STRAINS NO	TYPE	STRAINS NO	TYPE	STRAINS NO	TYPE	NO
3	2	4	A	10	7	1	1	10
2	3	1	B	1	8	1	2	
						1	5	
						1	6	
5		5		11		4		10
0.5%		0.5%		1.2%		0.4%		1.1%

## DISCUSSION

TABLE 71 ISOLATION OF POLIO VIRUS FROM CONTACTS  
EPIDEMIC OUTBREAKS IN INFANTILE COMMUNITIES OF BRESCIA (FROM TYPE 1)  
AND FLORENCE (FROM TYPE 2)

CITY AND YEAR	NO OF SUBJECTS EXAMINED	SUBJECTS WITH VIRUS IN STOOLS		TYPE OF VIRUS ISOLATED
		No	%	
Brescia 1953	Patients	7	70	1
	Contacts	65	50	1
	Staff	8	12	1
		5		
Florence 1955	Patients	2	100	2
	Contacts	17	76	2
	Staff	3	33	2
		13		
		1		

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2 Also in nonepidemic periods in absence of paralytic polio cases the virus causes inapparent infections in the population

3 Noteworthy is the isolation from healthy subjects and from infantile gastro-enteritis cases of Type 3 virus which in observed regions in these last years resulted rarely enough in paralytic polio cases. The presence of Type 3 virus as a latent infection agent may explain in part the high incidence of neutralizing antibodies in the healthy people an incidence which as has been noted is not much less than that found for Types 1 and 2

4 In contacts the latent abortive infections may reach 50 to 90 per cent and more

5 In contacts it is possible to show the presence of antibodies neutralizing the type of the virus causing the infection in 100 per cent of the cases

In conclusion the antipolio vaccination problem may be summarized as follows

1 The large exposure to the virus and thus the high incidence of spontaneous infections allow natural immunity to be reached by our population in their first years of life

2 As paralytic polio strikes with greater frequency children from 6 months to 3 to 4 years vaccination against poliomyelitis should be made at the beginning of the seventh month of life or during the following 5 months

3 Vaccination for all the children of these age groups even if desirable does not seem to be imminent in our country owing to economic and organization difficulties

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PROF OMER BLOM As Dr Payne pointed out the need for vaccination may be different even in different population groups in the same country. In Finland antibody studies have been performed which seem to show this clearly. I am therefore going to present a few illustrations which summarize some of these studies

Altogether about 2500 blood samples were collected in 1953 and 1954 in the regions mentioned in Figure 105 and were tested by Dr Lapinleimu and I myself in our laboratory. As was mentioned previously Finland has a poliomyelitis mortality ranging from 8 per 100 000 to about 70 in epidemic years. However some local epidemics with a far greater incidence have occurred (Fig 106). In such Type 1 epidemics happened to occur during or after the sampling in 3 out of the 5 regions shown.

The immunity against Type 1 virus in these regions is shown in Figure 107. I have tried to use the same method as Dr Payne in showing the number of susceptibles—probably not with good success—but the 50 per cent susceptibility is reached in Helsinki and the hemjars district at an age between 5 and 9 years, but on

- 1 The city of Helsinki
- 2 The Åland Islands
  - a The Khamo district
  - b The Jom district
  - c The Khamo district
  - d The Åland Islands



FIG 105 Districts investigated in 1953

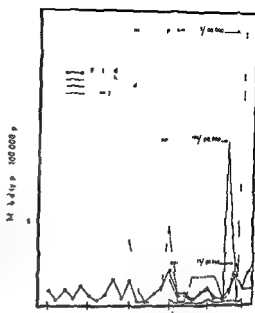


FIG 106 Annual rates per 100 000 population of notified cases of poliomyelitis (Finland Helsinki Åland Islands and Kemijärvi)

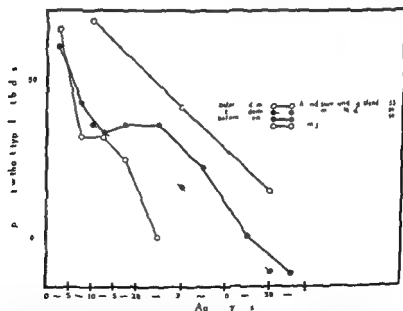


FIG 107 Frequency of persons without Type 1 poliomyelitis antibodies in Åland Islands Helsinki and Kemijärvi (Åland figures from Oker Blom and Strandsström Ann med exper et biol Fenniae 34 186 Helsinki and Kemijärvi figures from Lapinleimu Ann med exper et biol Fenniae 34 Supp 11)

Aland not until between 20 and 30 years. The 10 per cent susceptibility again was reached in Kemijarvi at an age of about 70 whereas it was reached in Helsinki at an age between 40 and 50 and on Aland not at all.

The age distribution of cases occurring during the epidemics which followed corresponded fairly closely to the curves in Figure 108. Thus in the Kemijarvi epidemic mostly children under 9 years were affected. In Helsinki the age distribution was similar to that in many other countries in Europe with a change toward higher age groups. On Aland there was a high incidence of cases in the upper age groups similar to the epidemics on for instance St. Helena described by Dr. Cear.

Thus there seems to be in our country apparently, very different demands for vaccination in different parts of the country. In some parts a vaccination of only children below 10 years would be enough whereas in other parts vaccination of everybody up to an age of 40 or 50 would be necessary.

Vaccine is not manufactured in Finland and for several reasons a large scale vaccination program cannot be carried out. Therefore it would be important for us to know in which regions and in which age groups the vaccination would be needed most urgently. For that reason another study of about 7500 blood samples has been initiated. This study was sponsored by the Presidentti Paasikivi Tohtori Wenner-Grenin tutkimusrahasto and samples were collected from different parts of the country by using random sampling methods worked out by Dr. Penttinen. The tests have been carried out in 3 laboratories and have been completed recently but unfortunately are not yet ready for presentation. However it is hoped that this study together with the already performed antibody studies and the epidemiologic data from different parts of the country may help the authorities to make plans for vaccination on a sound basis.

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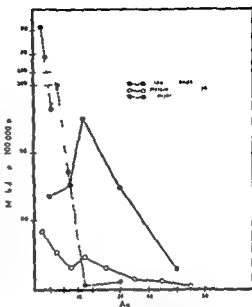


FIG. 109. Age incidence of poliomyelitis in Aland Islands (1953), Helsinki (1954) and Kemijarvi (1955) (Aland figures from Oker Blom. Ann med exper et biol Fenniae 34 10. Helsinki and Kemijarvi figures from Lapinleimu. Ann med exper et biol Fenniae 34 Supp. 11).

Oker Blom N. and Strandstrom H. Ann med exper et biol Fenniae 34 186 1956.

Dr. SALA. In view of the important question of the economics of immunization I asked Dr. Cear if he would let me present some slides which show a comparison in the antibody response after 2 doses of Reference Vaccine A. In the first instance (Fig. 109) the symbols on the left indicate the antibody response to 1 cc given subcutaneously; the symbols in the middle indicate the response to 1 cc, 1/4 cc or 1/16 cc given intramuscularly and the symbols on the right indicate the response to 1/10 cc given intradermally. If you look across the slide you will see that there is some advantage to the smaller dose when given by the intradermal route.

NOTE: Dr. SALA was not seen. He had led to the conclusion that the results of the study would be published in the near future.

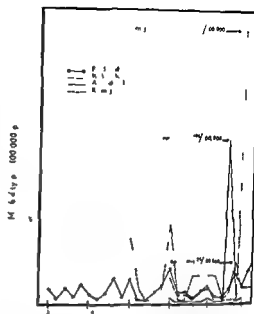
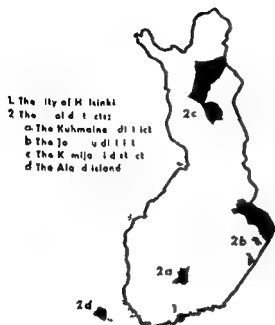


FIG 106 Annual rates per 100 000 population of notified cases of poliomyelitis (Finland Helsinki Åland Islands and Kemijärvi)

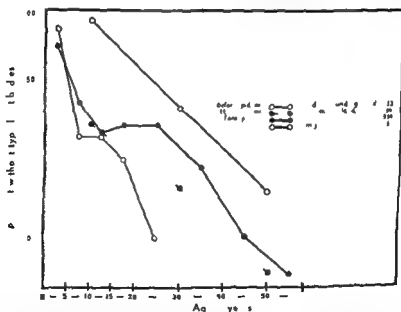


FIG 107 Frequency of persons without Type I poliomyelitis antibodies in Åland Islands Helsinki and Kemijärvi (Åland figures from Oker Blom and Strandström Ann med exper et biol Fenniae 34 186 Helsinki and Kemijärvi figures from Länimleimu Ann med exper et biol Fenniae 34 Supp 11)

Aland not until between 70 and 80 years. The 10 per cent susceptibility again is reached in Kemijarvi at an age of about 20 whereas it is reached in Helsinki at an age between 40 and 50 and on Aland not at all.

The age distribution of cases occurring during the epidemics which followed corresponded fairly closely to the curves in Figure 109. Thus in the Kemijarvi epidemic mostly children under 9 years were affected. In Helsinki the age distribution was similar to that in many other countries in Europe with a change toward higher age groups. On Aland there was a high incidence of cases in the upper-age groups similar to the epidemics on for instance St Helena described by Dr Cear.

Thus there seems to be in our country apparently very different demands for vaccination in different parts of the country. In some parts a vaccination of only children below 10 years would be enough whereas in other parts vaccination of everybody up to an age of 40 or 50 would be necessary.

Vaccine is not manufactured in Finland and for several reasons a large scale vaccination program cannot be carried out. Therefore it would be important for us to know in which regions and in which age groups the vaccination would be needed most urgently. For that reason another study of about 7500 blood samples has been initiated. This study was sponsored by the Presidentin Puolustusvoimien Terveystieteiden tutkimuskeskus. The samples were collected from different parts of the country by using random sampling methods worked out by Dr. Lintinen. The tests have been carried out in 3 laboratories and have been completed recently but unfortunately are not yet ready for presentation. However it is hoped that this study together with the already performed antibody studies and the epidemiological data from different parts of the country may help the authorities to make plans for vaccination on a sound basis.

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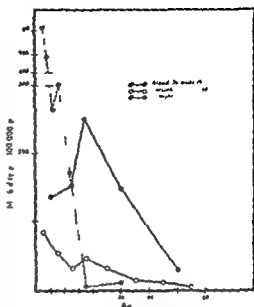


FIG 109 Age incidence of poliomyelitis in Aland Islands (1953) Helsinki (1954) and Kemijarvi (1955) (Aland figures from Oker Blom Ann med exper et biol Fenniae 34 170 Helsinki and Kemijarvi figures from Lapinleimu Ann med exper et biol Fenniae 34 Supp 11)

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Dr. SAA. In view of the important question of the economics of immunization I asked Dr. Cear if he would let me present some slides which show a comparison in the antibody response after 2 doses of reference vaccine A. In the first instance (Fig 109) the symbols on the left indicate the antibody response to 1 cc given subcutaneously, the symbols in the middle indicate the response to 1 cc, 1/2 cc or 1/16 cc given intramuscularly and the symbols on the right the response to 1/10 cc given intradermally. If you look across the slide you will see that there is some advantage in the smaller dose when given by the intradermal route.

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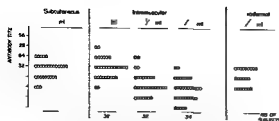


FIG 109 Different routes of inoculation Type 1 antibody response to Reference Vaccine A 2 weeks after 2 doses given 2 weeks apart

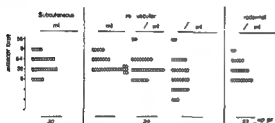


FIG 110 Different routes of inoculation Type 2 antibody response to Reference Vaccine A 2 weeks after 2 doses given 2 weeks apart

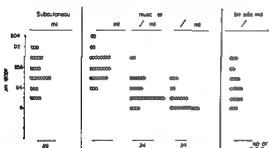


FIG 111 Different routes of inoculation Type 3 antibody response to Reference Vaccine A 2 weeks after 2 doses given 2 weeks apart

The next figure (Fig 110) shows similar data for the Type 2 response—you can exercise your own judgment in its interpretation.

Figure 111 shows the antibody response to Type 3. Here again we see the slight advantage to 1 cc given intramuscularly but it is clear that a small dose given intradermally seems to have a greater effect than a correspondingly small dose given by the intramuscular route.

The next figure (Fig 112) shows the results

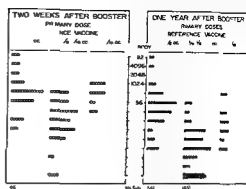


FIG 112 Comparison of intramuscular and intradermal routes for primary vaccination Distribution of Type 1 antibody titers following booster dose 1 year after primary (booster dose = 1 cc Vaccine J 1 m) (In this figure and Figures 113 and 114 the circles on the left indicate antibody levels 2 weeks after a constant booster dose in all groups where primary injections consisted either of 2 1/2, 1/4, 1/8 or 1/16 cc vaccine given 1 m or 1/10 cc given 1 d. Data for groups given 2 1/2 or 1/2 cc are considered together and groups given 1/4, 1/8 or 1/16 are similarly combined for comparison with groups given 1/10 cc 1 d. The solid dots on the right indicate antibody levels 1 year after the booster in the same groups.)

observed in these same persons who were given a booster dose intramuscularly with 1 cc of Vaccine J 1 year after the primary—if I may use the word—sensitization with 1/10 cc intradermally or to 1/4, 1/8, 1/16 intramuscularly or the larger dose of 2 1/2 or 1/2 cc intramuscularly. Two weeks after the booster dose the response was somewhat greater for those primarily sensitized with 1/10 cc intradermally as compared with those who had an average of about the same dose intramuscularly. The same persons were then bled 1 year after the booster dose without any further intervening inoculations. You will observe the extent of decline comparing the left and the right column and you will see that all of them had Type 1 antibody 1 year later. The sizes of the groups are indicated at the bottom. The smaller intramuscular dose was followed by a considerable decline with a number in the zero range in the interval of 1 year.

If one has vaccine of low potency such as an order of magnitude of 1/4, 1/8 or 1/16 cc and one

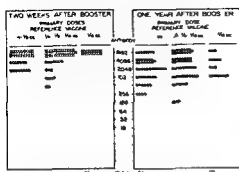


FIG 113 Comparison of intramuscular and intradermal routes for primary vaccination. Distribution of Type 2 antibody titers following booster dose 1 year after primary (booster dose = 1 cc vaccine i.m.)

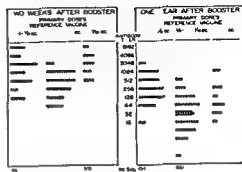


FIG 114 Comparison of intramuscular and intradermal routes for primary vaccination. Distribution of Type 3 antibody titers following booster dose 1 year after primary (booster dose = 1 cc vaccine i.m.)

then gives 1/10 cc of such vaccine intradermally one can expect a somewhat lesser effect intradermally than is shown here at the end of the year. While there is an advantage to the intracutaneous route when equal volumes are administered the fact that one can give a larger volume intramuscularly provides some slight advantage in compensating for weak vaccine.

What this means is another matter. I refer strictly to the numerical value for antibody. Figure 113 shows the Type 2 response here again the very high order of magnitude. This is perhaps the super vaccine to which someone referred today.

The last figure (Fig 114) shows the Type 3 response indicating again the advantage and also the comparisons.

Dr. PALL: Certainly Dr. Payne has shown us how different the problems are and the rest of the members of this panel have also indicated how the different factors of incidence, age groups and levels of society are involved in poliomyelitis. This clearly means that the problems of immunization are different in these various places. The panel has agreed that the formalized vaccine is desirable that it can be made safely and that its use is to be recommended especially in inter epidemic or so-called pre-epidemic periods although now it appears also that during epidemics the vaccine should be used.

The cost of this vaccine is high. If it was not high we would not have needed this session this

afternoon because much time has been spent on seeing how it should be rationed. There may be some countries even which will wish to wait and try the use of an attenuated vaccine in the hope that at least a field trial will be made so that its value could be assessed. There was a suggestion made this morning in favor of another Francis field trial and all that goes with it to give some idea as to the value of an attenuated vaccine.

Dr. Payne's Groups 1 and 2 were in the ideal for this particular sort of trial. However some of us rabid epidemiologists look with alarm on the disappearance of nonvaccinated populations. We have to get our controls somewhere and we will try to keep our eyes open for them in the last few years before they are gone.

Granted that the vaccine is desirable though expensive and in short supply some countries should make their estimates not only on the basis of past experience but also on predictions as to what might happen in the next decade. In some countries local cases are still few and epidemics are unknown. It might appear that such countries would be ill-advised to go into any major or expensive vaccination program in view of the meager returns. However I hope that it is not presumptuous to point out and Dr. Chumakov mentioned this also that countries can have a rude awakening such as happened in Costa Rica in January in the British West Indies where within the past 3 years poliomyelitis epidemics of great severity have appeared precipitously with an abrupt change from the infantile paralysis of

our parents and grandparents to that of modern epidemic polio. And once modern poliomyelitis has appeared the subsequent pattern seems to be an irreversible one unless perhaps it is influenced by artificial immunization.

As long as I am putting myself in the unenviable position of a prophet, what are the diagnostic signs in some of these countries which feel secure at the moment with low rates of poliomyelitis but might be on the eve of becoming afflicted with modern epidemic poliomyelitis? First I think we should turn to features other than antibody studies, namely let us not forget about the changing ways of life in various places and the rather crude indication of the record of infant mortality. At the last International Poliomyelitis Conference Dr. Payne presented this thesis: that low infantile mortality rates were often associated with high poliomyelitis rates, and he insisted that there was no cause or relationship which I think is correct although his data testified just as eloquently that there is an extraordinary association. In some of these countries in which the infant mortality rate is dropping from 100 to 75 this trend would seem to be a preliminary indication that something might happen.

A second feature is the study of the age distribution of the cases. It may seem foolish in many places to try to analyze statistically the age distribution of a handful of cases, and yet as early as 6 or 8 years before the most recent epidemic in Jamaica the shift began, and when the figure of 20 per cent over the age of 10 appeared I think it was time to be watchful.

Finally we come to the question of the antibody surveys and the extent to which they may be used as an indication. We should not lay too much stress on that particular aspect, valuable as the surveys are and perhaps the only measurement that could be made. When it comes to interpreting the results of the antibody surveys, one may recognize that it is not the antibody level alone that indicates whether the population is immune or ripe for an epidemic. It is probably the ratio between antibody level and an unknown figure which represents dosage or availability of virus or amount of virus. What would be a normal level of antibody for school children in the cities of the United States? As Dr. Melnick has shown, might be a low level in a tropical area where in terms of dosage of the polio virus to which the population is exposed it might be

much higher. I think we should recall Dr. Gears' remarks made at previous Congresses that poliomyelitis may be essentially a tropical disease, perhaps based on the fact that facilities for its spread do exist in tropical countries to a degree that may not be true elsewhere.

I do not want to belittle the surveys but I am sure that the antibodies are not everything. We heard much this morning about the possibility of local immunity, something that cannot be measured but certainly should be dealt with. The only thing I would suggest in the planning of the use of the Silk vaccine is that it should be remembered that one of the most characteristic features of this particular disease is its tendency to change.

**DR. HENNEBERG.** Following Dr. Payne's suggestion it is more correct to speak of susceptibility rather than of immunity, even though this concept is not unambiguously characterized by lack of neutralizing antibodies against the 3 types of poliomyelitis virus. In serology positive specific reactions are better indicators than negative ones, since the latter depend strongly on reaction threshold values which fluctuate from day to day. In the neutralization test a serum which is still positive at a serum dilution of 1:4 should be used concurrently as a control.

### INDICATIONS FOR INOCULATION

Generally valid statements about an epidemic situation in a large area can scarcely be made; this can be done only for a narrowly limited time and place.

Ever since 1945 West Berlin has been in a situation unusual for a metropolis. The usual fluctuations of population have been absent; contacts with the environs have been limited greatly, and in brief everything which admittedly serves as a starting point for virus dissemination has been minimized. In addition the reconstruction of the city automatically eliminated unhygienic conditions. Since the epidemic of 1947 and the postepidemic conditions of 1948-1949 there has been no poliomyelitis epidemic in Berlin which might have served to spread the polio viruses, and consequently the prerequisites for epidemic contamination have been lacking. The data presented below should be regarded in this light. The morbidity included both paralytic and non-paralytic cases, which was possible in Berlin because clinically speaking all cases charted were

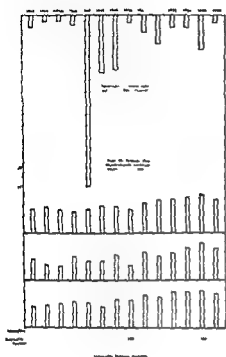


FIG. 115. Morbidity of poliomyelitis in Western Berlin as function of the number of persons without neutralizing antibodies (up to 1945 Berlin; after 1946 Western Berlin).

**POLIOMYELITIS INCIDENCE**  
PER YEAR PER 100 000 INHABITANTS  
From 1940-1945 for Berlin (per 3 500 000)  
From 1946-1956 for West Berlin (per 2 300 000)

Year	Total Incidence	Per 100 000 Inhabitants	
1940	63	1.8	
1941	4.4	13.5	
1942	763	7.5	
1943	143	4.0	Berlin
1944	61	1.7	
1945	55	1.6	
1947-51 12			
1946	45	3.0	
1947	1255	54.0	
1948	418	18.0	
1949	404	1.1	
1950	51	2.0	
1951	115	5.0	West Berlin
1952	209	9.0	
1953	85	4.0	
1954	86	4.0	
1955	244	10.6	
1956	54	2.3	

diagnosed as poliomyelitis considering the differential diagnosis involved. The limitation of the morbidity index to paralytic cases only, which Payne considers necessary in a clinical investigation, is a procedure dictated solely by necessity. The statistical classification of a single definite degree of severity of a disease, even of a special case of the disease, is unusual, especially since we know that various internal and external factors have a decisive influence on the clinical picture.

We show the relation of morbidity per 100 000 for the years 1943-1956 to the negative neutralization tests expressed as percentages of the subjects tested. The figures are classified by age classes 0 to 14 years and by virus types.

In 1941-1949 an epidemic of major proportions swept over all of Berlin. The effect of the pandemic—which was reported by Salin to be of Type 1—was reflected in the percentage susceptible. Children born before or during the epidemic exhibit a lower percentage of susceptibility than those born later. However, the differences are so very slight that we cannot really

speak of an unusual alteration in percentage negative by a wave of immunization emerging during the epidemic in the age classes concerned. The percentage susceptible to Type 3 apparently was not affected, increasing at a constant rate throughout the year of the epidemic.

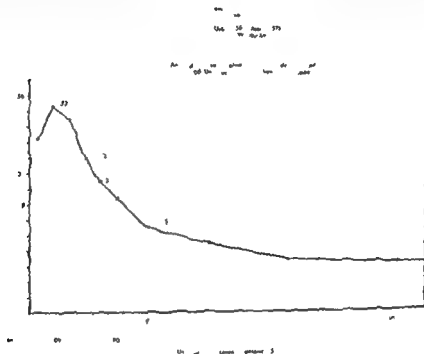
Those susceptible to all 3 types were classified by age or age groups. The constant and relatively high percentage negative to all 3 types fell mainly in the higher age classes, being about 18 per cent in the age group of 9 to 19 years and 17.5 per cent in the group 30 years and older. With regard to the present epidemic situation and the concomitant virus dissemination to be expected, we may predict that the number of persons without antibodies will become larger as the younger age groups grow older.

I think it would be better not to picture the situation as is usually done in the form of a graph. To do so would be to prejudice a development which need not necessarily appear throughout. In regard to convenience of exposition, we may assume that the percentage of susceptible

in the cohort which is now 14 years old remains the same in this cohort as it ages

In order to get an idea of the effectiveness of immunization throughout definite professional groups the medical personnel were tested in the clinic. The relatively high proportion of Type 1 susceptibles is astonishing amounting to 26 anti body negatives in a total of 67 people. Also note

worthy is the distribution of high antibody titer (1:512 and higher) among workers in the polo and the typhus laboratories and among mothers with children under 10. In 23 of these subjects there were 17 with a neutralization test for 1 of the 3 virus types of 512 or higher while the 31 members of the Institute staff all had titers below 512.



NUMBER OF PERSONS WITHOUT NEUTRALIZING ANTIBODIES  
FOR TYPES 1, 2 OR 3

AGE	PERSONS EXAMINED	1		2		3	
			%		%		%
0-1	99	72	72.7	67	67.6	72	72.7
1-2	109	87	80	87	80	85	78
2-3	109	86	78.9	76	69.7	84	77
3-4	116	85	73.2	65	56	89	76.7
4-5	89	62	70	46	51.2	57	64
5-6	90	57	63.3	54	60	61	67.7
6-7	100	51	51	32	32	57	57
7-8	90	51	56.7	49	54.4	54	60
8-9	74	43	58.1	29	40	33	44.6
9-10	59	29	50	24	40.7	31	52.5
10-11	55	24	43.6	27	50	29	52.7
11-12	59	29	47.4	18	30.5	30	50.8
12-13	71	39	53.5	23	32.4	33	46.5
13-14	55	28	50.9	24	43.6	23	41.8

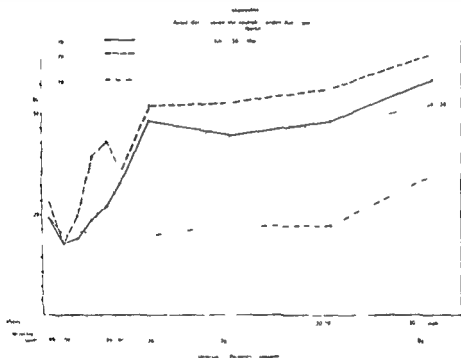
Total 1175 Persons Examined

FIG. 116

In Berlin the number of persons with incomplete immunity seems to be increasing, placing the city in the group which Dr Layne has named the Melbourne Group. The statistical data show no decrease in antibodies with increasing age, an observation however which may not be valid beyond the present situation. A loss of immunity would manifest itself in the appearance of second

cases of the disease among the older age groups.

In addition when I found only 78 paralytic second cases of poliomyelitis in the literature (Dagulf) to date this small number of cases took on significance. Perhaps they may serve to shake the false security against recurrence of the disease which is now so widespread. The conditions under which paralytic polio due to 1 of the 3



POLIOMYELITIS  
NUMBER OF PERSONS WITH NEUTRALIZING ANTIBODIES IN WEST BERLIN

Age	Persons Examined	1	2	3	1 + 2 + 3
0-10	99	2	32	2	6
1-11	109	2	31	24	4
1-11	109	3	30	5	5
1-11	116	31	51	5	9
1-11	87	30	43	3	12
1-11	90	33	36	2	8
1-11	74	144	154	10	58
1-11	365	187	21	193	29
1-11	174	95	110	91	43
30 and over	104	48	5	61	40

Total 15141 persons examined

types of polio virus develops are so varied that the probability of developing poliomyelitis a second time is in principle very small

If the statement that people living in a polio virus infected environment or subjects in contact with poliomyelitis patients, polio virus secretors or poliomyelitis virus cultures have a lower antibody deficiency and an increased antibody titer is true, this observation could make it necessary to take into account simultaneous dissemination of polio viruses when the effect of artificial immunization is evaluated. The inoculation of a popula-

tion which has only infrequent and slight contact with polio viruses can lead merely to a shorter immunization than if the dissemination of the polio viruses brings with it more frequent contacts thereby leading to a stimulation effect. In the type of case first mentioned (that of Berlin for example) involving limited dissemination of virus several timely vaccinations during a person's life time as in the case of smallpox should be considered. If polio protective vaccination is once introduced, continuation of such vaccination in future years will have to be planned, not haphazard.

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# Enteric Viruses Producing Disease Simulating Poliomyelitis

TUESDAY AFTERNOON, JULY 9, 1957

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## *Chairman*

PROF DR C H STUART HARRIS

Department of Medicine  
Royal Hospital  
Sheffield England

## *Speakers*

### ECHO VIRUSES

DR JOSEPH L MINICK

College of Medicine  
Baylor University  
Houston

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Department of Bacteriology and Immunology  
University of Buffalo  
Buffalo

DR HERBERT A WENNER

Department of Pediatrics  
University of Kansas Medical Center  
Kansas City Kan

### COXSACKIE VIRUSES

DR GILBERT J DAVIDOFF

State of New York  
Department of Health  
Albany

DR MICHAEL L FURCOLOW

U.S. Public Health Service  
Kansas City Kan

DR JAMES H SCAR

South African Institute for Medical Research  
Johannesburg

## *Discussants*

Moderator PROF DR van COTTEN

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DR WILLIAM McD HAMMON

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DR TORSTEN JOHNSON

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Stockholm

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DR ARNE SVEDMYR

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Stockholm

PROF DR J D VERLINDE

Netherlands Institute for Preventive Medicine

Leiden

# The ECHO Viruses and Their Classification

DR JOSEPH L. MELNICK

As soon as tissue-culture methods began to be applied to the isolation of viruses it became obvious that viruses other than poliomyelitis and the Coxsackie viruses existed in the human enteric tract. It early became apparent that these agents could be isolated from normal children as well as from patients with the aseptic meningitis syndrome and that multiple antigenic types exist. As more investigators entered the field more and more of these untypable agents came to be recognized especially in those laboratories using monkey kidney cultures. Because the human diseases to which they belonged were unknown and because they failed to produce illness in laboratory animals these agents were called the orphan viruses.

A working committee formed to facilitate the classification of these new agents found what it believed to be a more suitable name for these viruses and proposed that they be called the enteric cytopathogenic human orphan or ECHO viruses. A co-operative study on the prototype strains then available (8 in our laboratory, 5 in Dr Salinas and 3 in Dr Hammon's) resulted in the differentiation of 13 antigenically distinct viruses. None of the original 13 prototype viruses or the 6 or 7 new ones which have been identified subsequently had been found to multiply and produce disease in laboratory animals. However there may be some need to qualify this aspect of the definition of ECHO viruses in view of some recent observations on which comment will be made later.

The laboratory data required to establish a strain as a new prototype are shown in Table 7. Here we can see the data required to establish the Tow strain as the prototype strain of No. 14. In addition to the failure of the Tow strain to be neutralized by the antisera shown these antisera being effective against the other known ECHO viruses in listing, each of the previously known types a new serum had to be prepared which was specific for the virus in that the pre-immunization serum was negative but the

postimmunization serum contained antibodies for this type alone. A more or less uniform technic was adopted. A new prototype was established as one which at a test dose of 100 tissue-culture doses failed to be neutralized by 70 units of antibody against each of the previously known virus types conversely its serum at a concentration of 20 units failed to neutralize each of the other virus types. Because ECHO mixtures may occur in nature the ECHO Virus Committee has recommended that all sera to new types be prepared with virus particles derived from a single clone obtained either from a plaque or from a terminal dilution culture.

Neutralization tests as shown in Table 72 were also conducted with paired serum specimens obtained from the patient from whom the virus was recovered. In accordance with the

TABLE 72. NEUTRALIZATION TESTS ESTABLISHING TOW STRAIN AS ECHO TYPE 14

A. SPECIFIC NEUTRALIZATION OF TOW STRAIN	
SERA	TITERS AGAINST 100 T.C.D. <sub>50</sub> OF TOW VIRUS
Monkey No. 8076	
Preimmunization serum	<10
Postimmunization with Tow strain	370
Latent Tow	
4th day after onset	<10
93rd day after onset	100
B. TOW VIRUS (3.2 TO 100 T.C.D. <sub>50</sub> ) NOT NEUTRALIZED BY	
Antisera to poliomyelitis Types 1, 2, 3	
Antisera to Coxsackie virus Types	
A 1 2 3 4 5 6 7 8 9 10 11 14	
B-1 2 3 4 5	
Antisera to ECHO virus Types	
1 2 3 4 5 6 7 8 9 10 11 12 13	

At a titer of 1:10 the post-immunization serum failed to neutralize ECHO viruses 1 through 13.

TABLE 73 SPECIFICITY OF ECHO TYPE 14 (TOW STRAIN) IN THE COMPLEMENT FIXATION REACTION

SERUM	COMPLEMENT FIXING ANTIBODY TITER AGAINST				
	ADENOVIRUS ANTIGEN	POLIO ANTIGENS			TOW VIRUS ANTIGEN <sup>a</sup>
		TYPE 1	TYPE 2	TYPE 3	
Tow monkeys					
No 8017					
Preinoculation serum	<8	<8	<8	<8	<8
Postinoculation serum	<8	<8	<8	<8	13
No 8076					
Preinoculation serum	<8	<8	<8	<8	<8
Postinoculation serum	<8	<8	<8	<8	128
Adenovirus antiserum	> 128	<8	<8	<8	<16
Polio virus antiserum					
Type 1	<30	240	<30	<30	<30
Type 2	<30	<30	120	<30	<30
Type 3	<30	<30	<30	240	<30

<sup>a</sup> Tissue culture fluid at a dilution of 1:2 which contained 8 units of antigen

principles of classical immunology the patient developed neutralizing antibodies during his convalescence

Complement fixing antibodies also appeared in monkeys inoculated with the Tow strain of virus (Fig 118) The CF tests listed in Table

73 indicate that the antibodies were specific for the new type They fail to cross with the adenoviruses or with the polio virus antigens

I mention here that all is not quite as simple and clear as it might seem For example as shown in Table 74 certain ECHO viruses cross-

TABLE 74 RELATIONSHIPS AMONG ECHO VIRUSES 6, 6 AND 6

VIRUS (100 TCD)	SERUM TESTED			
	TYPE 6 RH 7819	TYPE 6 CHIMP 45	TYPE 6 RH 8922	TYPE 6 RH 8789
	A. SERUM TITER			
Type 6 (D Amori)	320	64 000	600	60 000
Type 6 (DiMeo)	10	150	300	2 400
Type 6 (Burgess)	11	30	20	2 100
	B. NEUTRALIZATION RATIO †			
Type 6 (D Amori)	10	10	20	286
Type 6 (DiMeo)	0.03	0.002	10	11
Type 6 (Burgess)	0	0.0005	0.07	10

0—titer of less than 10

† Neutralization Ratio =  $\frac{\text{Titer against heterologous virus}}{\text{Titer against homologous virus}}$   
(11 ml of serum arbitrarily given value of 10)

react but chiefly in one direction. Strains which have a broader antigenic composition than the prototype strains have been classified provisionally as prime strains. Thus some strains have been recognized which are related to but are not identical with the prototype strain of Type 6. These 6 strains produce antisera which neutralize ECHO-6 prototype strain; the 6 strains are not neutralized by Type 6 antisera unless the sera have a high Type 6 antibody titer as shown by the serum of the chimpanzee in the second column which partially neutralizes Type 6. Type 6 strains seem to have even broader antigenic overlap with the prototype ECHO-6 strain in that their antisera manifest a higher titer against ECHO 6 than against the homologous virus used to immunize the monkey. Antigenic variance exists among other types also notably among Types 5, 9 and 11.

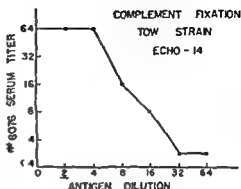


Fig. 118 Complement fixation box titration of Tow antiserum from monkey No. 8076 against Tow antigen. The tissue-culture fluid was used at a dilution of  $1:2$  which contained 8 units of antigen.

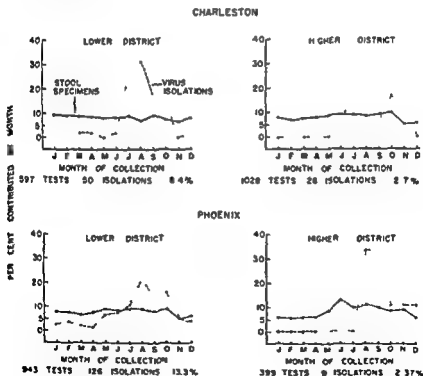


Fig. 119 Stool collections and enteric virus isolations cumulative for each month over a 3-year period (1951-1953). Specimens were obtained from healthy children in contrasting socioeconomic strata of Charleston, W. Va., and Phoenix, Ariz. during a nonepidemic period.

Recent studies by members of the Committee on the ECHO Viruses have shown that the prototype ECHO 1 and ECHO 13 strains may have to be reconsidered as belonging to distinct types. It had been found that the ECHO 1 typing serum which we had originally prepared when the virus was first identified failed to neutralize a new prototype strain isolated in Dr Hammon's laboratory. This particular strain had been designated as ECHO 13. New anti sera against ECHO 1 have been prepared and they have been found to neutralize not only ECHO 1 but also ECHO 13 virus conversely new ECHO 13 antisera prepared with later passages of the virus now neutralize ECHO 1 virus. The full details of these experiments which will be published as a Committee Report suggest that ECHO 13 is a mixture of two viruses ECHO 1 plus an antigenically distinct ECHO 13 or perhaps that ECHO 1 shows a prime relationship to ECHO 13.

I turn now to the epidemiology of the ECHO

viruses. Several laboratories in many countries are conducting studies on the viral flora of the human enteric tract. We in conjunction with the Epidemiology Branch of the Communicable Disease Center have recently completed a longitudinal study of the endemic occurrence of enteric viruses among several hundred normal households in Charleston, West Virginia and Phoenix, Arizona. Some relevant findings indicate what may be learned from such studies. Approximately equal numbers of stools were collected each month from normal children living under contrasting environments. However, almost all of the virus isolations were made in the summer and the fall except from the lower socioeconomic district of Phoenix (Fig. 119) where viruses were recovered throughout a larger part of the year. Figure 120 illustrates the repeatable seasonal incidence of enteric virus excretion among normal children.

The frequency of virus excretion in the lower socioeconomic district of each city was 3 to 6

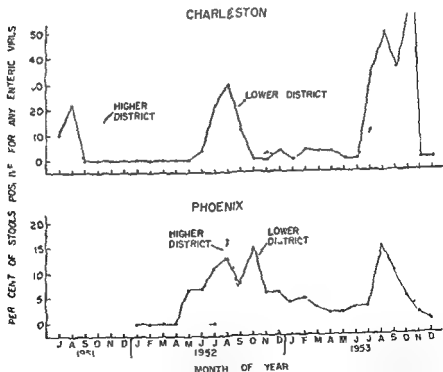


FIG. 120. Data of Figure 119 arranged to show the per cent of stool specimens yielding enteric viruses by month through the study period in higher and lower socioeconomic districts.

TABLE 5 PER CENT DISTRIBUTION OF ENTERIC VIRUSES ISOLATED FROM HEALTHY CHILDREN IN POPULATIONS OF CONTRASTING SOCIOECONOMIC LEVEL DURING A NONEPIDEMIC PERIOD (1951-53)

POPULATION GROUP	NUMBER OF SPECIMENS TESTED	PER CENT YIELDING VIRUSES			
		PC 10 VIRUSES	Coxsackie Viruses	ECHO Viruses	ALL ENTERIC VIRUSES
Charleston W. Va.					
Lower	597	7.3	2.3	3.7	8.4
Upper	1,028	0.5	1.5	0.8	2.7
Phoenix Ariz.					
Lower	943	3.0	7.0	8.3	13.3
Upper	399	1.0	1.0	0.3	2.3
Total					
Lower	1,540	7.8	2.1	6.6	11.4
Upper	1,427	0.6	1.3	0.6	2.6

times as great as in the middle to upper middle class districts with good environmental sanitation. If the combined figures are examined here I call attention to the bottom two rows of Table 75—of 1,540 specimens tested in the lower districts 11.6 or 11 per cent were positive whereas in the upper districts only 2.6 per cent—31 of 1,427 specimens—yielded virus. Among the 713 viruses isolated in monkey kidney cultures 57 per cent were ECHO viruses, 7.4 per cent were polio viruses and 7.4 per cent were Coxsackie viruses.

#### ASSOCIATION OF ECHO VIRUSES WITH ASEPTIC MENINGITIS

Although some ECHO viruses have been recovered from patients with aseptic meningitis

initially indistinguishable from nonparalytic polio the epidemiologic criteria regarding all of the ECHO viruses as etiologic agents of this syndrome have not yet been met. However certain members have been associated with aseptic meningitis outbreaks and certain types notably Types 5, 6, 9, 14 and perhaps others have been isolated from the cerebrospinal fluid of aseptic meningitis patients in the laboratories of Hodges, Karzon, DeSommer, as well as others.

The experience with aseptic meningitis cases in Connecticut during the past 2 years is summarized in Table 76. It can be seen that ECHO Type 6 strains and the Coxsackie viruses of Types B<sup>1</sup>, B<sup>3</sup>, B<sup>4</sup> and A<sup>9</sup> were as frequent contributing factors in aseptic meningitis as were polio viruses. Over the 2 year period

TABLE 76 DISTRIBUTION OF ENTERIC VIRUS ISOLATIONS FROM ASEPTIC MENINGITIS CASES IN CONNECTICUT DURING 1952 AND 1953\*

VIRUS ISOLATED	NUMBER OF ISOLATIONS		
	FROM 13 CASES STUDIED IN 1952	FROM 8 CASES STUDIED IN 1953	FROM TOTAL OF 21 CASES
Polio virus	5	5	10
ECHO 6	1	11	12
ECHO 14	0	0	0
Coxsackie A <sup>9</sup>	1	3	4
Coxsackie B <sup>1</sup> , B <sup>3</sup> , B <sup>4</sup>	15	4	19
Unidentified virus	0	4	4
Total virus isolation	21	27	48

\* Data from J. A. Melnick, J. Biol. Med. 59: 1, 1956 and J. A. Melnick, J. Biol. Med. 60: 1, 1957.

TABLE 77 ANTIBODY TITERS TO ECHO-6 VIRUS AND THE POLIO VIRUSES OBSERVED IN PAIRED SERA OF ASEPTIC MENINGITIS PATIENTS EXCRETING ECHO-6 VIRUS

PATIENT NO	SERUM	ECHO 6 NEUTRALIZING ANTIBODY TITER	POLIO VIRUS ANTIBODY TITER					
			NEUTRALIZING			COMPLEMENT FIXING		
			1	2	3	1	2	3
1	Acute	0	0	0	64	0	0	0
	Conv	100	0	0	64	0	0	0
2	Acute	0	256	0	0	0	0	0
	Conv	100	256	0	0	0	0	0
3*	Acute	0	256	1 024	1 024	0	0	0
	Conv	50	256	1 024	1 024	0	0	0
4*	Acute	10	64	1 024	16	0	4	0
	Conv	100	64	1 024	16	0	4	0

Patients 3 and 4 had been vaccinated against poliomyelitis some months before they became ill with aseptic meningitis. Their vaccination rather than natural infection probably accounts for the high level of neutralizing antibodies to all 3 types of poliovirus and the lack of CF antibodies children in Connecticut at the ages of these patients (6 and 8 years) rarely have had natural infections with all 3 types.

which is summarized in the last column on the table there were 32 polio virus isolations, 32 isolations of ECHO 6 and 28 isolations of the Coxsackie viruses listed from the total of 221 aseptic meningitis cases studied. Considering only those cases from which any virus was isolated, polio viruses and ECHO 6 each accounted for approximately 30 per cent of the total isolations, with the Coxsackie viruses accounting for about 27 per cent. Results of serologic tests on 4 patients excreting ECHO 6 virus are shown in Table 77.

A number of strains of a single type recently isolated by workers in England, Belgium, Hol-

land, Denmark, Germany, Italy, and Canada are worthy of special comment. A number of isolations were made by McLean from an epidemic of aseptic meningitis in Bourne, England. We had the privilege of studying this material and were able to identify the virus as ECHO 9. However, I would like to call attention to the incomplete crossings obtained with the Bourne strain from England, for example, as shown in Table 78 in the same test using the same lot of tissue-culture tubes, two established strains of ECHO 9 (Hill and Quigley) along with the Bourne strain from England were used to titrate the neutralizing capacity of their respective sera. While

TABLE 78 CROSS RELATIONSHIPS BETWEEN ESTABLISHED ECHO 9 STRAINS AND THE BOURNE STRAIN

VIRUS	SERUM TITER AGAINST 100-320 TCD <sub>50</sub> OF VIRUS			NEUTRALIZATION RATIO*		
	HILL ANTISERUM	QUIGLEY ANTISERUM	BOURNE ANTISERUM	HILL ANTISERUM	QUIGLEY ANTISERUM	BOURNE ANTISERUM
Hill	15 600	2 500	900	1 00	1 67	0 11
Quigley	15 600	1 500	3 400	1 00	1 00	0 40
Bourne M <sub>H</sub> †	5 400	220	8 500	0 35	0 15	1 00
Bourne M <sub>H</sub> in ‡	7 800			0 50		

Neutralization Ratio =  $\frac{\text{Titer against heterologous virus}}{\text{Titer against homologous virus}}$

† M<sub>H</sub>—Virus used after 6 passages in monkey kidney

‡ M<sub>H</sub> in—Virus used after 6 passages in monkey kidney plus 2 passages in infant m

there was complete crossing between the Hill and the Quigley strains both of these strains having been isolated in the United States the Bourne strain from England although antigenically related had a distinctive antigenic character. It was poorly neutralized by the Quigley antiserum and in turn as shown in the last column of Table 78 the Bourne antiserum gave a tenfold higher titer against the homologous strain than it did against the Hill prototype

aseptic meningitis some cases of which were similar to the disease observed earlier in babies in East London 3 years ago ECHO 9 viruses were isolated in tissue culture by both groups of workers. The same virus was isolated repeatedly from cerebrospinal fluids as well as from throat washings and feces during the 1956 aseptic meningitis epidemic which occurred throughout Europe notably in England Denmark Belgium Holland Germany and also in Italy

The Bourne virus was associated with a sharp epidemic of frank aseptic meningitis and subsequently with two outbreaks characterized by milder symptoms. Since ECHO-9 was the only virus isolated from the patients studied it seems reasonable to infer that this virus caused each outbreak. Rising titers of neutralizing antibody to current strains of ECHO 9 further strengthen this view. From these results it would appear that ECHO 9 virus as well as ECHO-6 must be added to the list of agents which may cause the aseptic meningitis syndrome. Support of this view has recently come from several sources. A virus isolated in monkey kidney cells by Dr DeSommer in Louisiana from the spinal fluid of an aseptic meningitis patient and sent to New Haven for typing also turned out to be an ECHO 9 virus. DeSommer isolated the same virus from the spinal fluids of ~ cases of aseptic meningitis and from 76 fecal specimens. Serologic studies showed eightfold or greater antibody increases during convalescence. Tyrrell and Snell in England have recently described an epidemic disease characterized by rash and

In addition to the association of ECHO 9 with outbreaks of aseptic meningitis and its recovery from the cerebrospinal fluid one other important fact has come out of these studies namely that certain strains of ECHO 9 virus after several passages in monkey kidney tissue produce paralysis and myositis in infant mice typical of the Group A Coxsackie viruses. The mouse virus has a high titer in tissue culture and in mice. ECHO 9 prototype serum when titrated in tissue culture against the prototype Hill strain the Quigley strain the Bourne strain or in mouse passage gave similar titers. As Table 79 shows at a dilution of 1 to 100 the serum neutralized 1 000 000 tissue-culture doses of virus grown in mice for 2 passages and then used as seed for the neutralization test. That the mouse pathogenic virus was identical with the virus grown in tissue culture was shown by neutralization tests in mice (Table 9 second column). The prototype Hill serum also at a dilution of 1 to 100 neutralized over 10 000 mouse paralytic doses of virus. The question

TABLE 9 NEUTRALIZATION TEST ON SERA TAKEN DURING THE BOLRAE EPIDEMIC OCTOBER 1955

PATIENT NO	SERUM TITER AGAINST		SERUM TITER AGAINST	
	MUSCLE PATHOGENIC BOLRAE STRAIN	CONVASCENT	MUSCLE PATHOGENIC QUIGLEY STRAIN	CONVASCENT
1	0	>100	0	50
3	0	40	0	50
4	0	50	0	50
5	10	>50	0	50
6	50	>50	0	50
	50	>50	0	100
	10	>50		



TABLE 77 ANTIBODY TITERS TO ECHO-6 VIRUS AND THE POLIO VIRUSES  
OBSERVED IN PAIRED SERA OF ASEPTIC MENINGITIS PATIENTS EXCRETING ECHO-6 VIRUS

PATIENT NO	SERUM	ECHO 6 NEUTRALIZING ANTIBODY TITER	POLIO VIRUS ANTIBODY TITER					
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			1	2	3	1	2	3
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	Conv	100	0	0	64	0	0	0
2	Acute	0	256	0	0	0	0	0
	Conv	100	256	0	0	0	0	0
3*	Acute	0	256	1 024	1 024	0	0	0
	Conv	50	256	1 024	1 024	0	0	0
4*	Acute	10	64	1 024	16	0	4	0
	Conv	100	64	1 024	16	0	4	0

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Bourne MK <sub>1</sub> †	5 400	220	8 500	0 35	0 15	1 00
Bourne MK <sub>1</sub> in ‡	7 800			0 50		

Neutralization Ratio =  $\frac{\text{Titer against heterologous virus}}{\text{Titer against homologous virus}}$

† MK<sub>1</sub>—Virus used after 6 passages in monkey kidney

‡ MK<sub>1</sub> in—Virus used after 6 passages in monkey kidney plus 2 passages in infant mice

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aseptic meningitis some cases of which were similar to the disease observed earlier in babies in East London 3 years ago. ECHO 9 viruses were isolated in tissue culture by both groups of workers. The same virus was isolated repeatedly from cerebrospinal fluids as well as from throat washings and feces during the 1956 aseptic meningitis epidemic which occurred throughout Europe notably in England Denmark Belgium Holland Germany and also in Italy.

In addition to the association of ECHO 9 with outbreaks of aseptic meningitis and its recovery from the cerebrospinal fluid one other important fact has come out of these studies namely that certain strains of ECHO 9 virus after several passages in monkey kidney tissue produce paralysis and myositis in infant mice typical of the Group A Coxsackie viruses. The mouse virus has a high titer in tissue culture and in mice. ECHO 9 prototype serum when titrated in tissue culture against the prototype Hill strain the Quigley strain the Bourne strain from England either in monkey kidney passage or in mouse passage gave similar titers. As Table 79 shows at a dilution of 1 to 100 the serum neutralized 1 000 000 tissue-culture doses of virus grown in mice for 2 passages and then used as seed for the neutralization test. That the mouse pathogenic virus was identical with the virus grown in tissue culture was shown by neutralization tests in mice (Table 79 second column). The prototype Hill serum also at a dilution of 1 to 100 neutralized over 10 000 mouse paralytic doses of virus. The question

TABLE 79. NEUTRALIZATION TESTS ON SERA TAKEN DURING THE BOURNE EPIDEMIC, OCTOBER 1955

PATIENT No.	SERUM TITER AGAINST			
	MOUSE PATHOGENIC BOURNE STRAIN		MOUSE PATHOGENIC QUIGLEY STRAIN	
	ACUTE	CONVALESCENT	ACUTE	CONVALESCENT
1	0*	>100	0	250
2	III	50	0	100
3	0	750		
4	10	750		
5	50	>250		
6	50	250		

\* Indicates 1 in 10

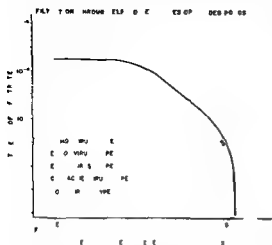


FIG 121 Correlation of pore size of membrane and filtrability of ECHO virus Types 1, 2 and 3, Coxsackie virus Type A9 and polio virus Type 1 all grown in monkey tissue culture

must be raised at this time—although it cannot be answered as yet—as to whether ECHO-9 really belongs with the ECHO viruses or with the Coxsackie viruses. If many, perhaps the majority of strains remain nonpathogenic for mice even after repeated attempts with virus carried serially in tissue culture, then this raises a difficult problem in classification. If the scheme of classification rests on the host strains of virus, then the host strains of the natural virus before

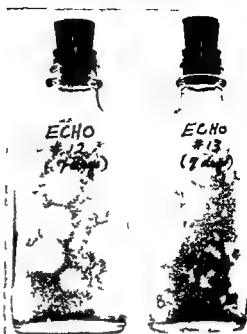


FIG 123 Representative plaques of the two groups of ECHO viruses (Left) Type B—ECHO 12 (Farouk) Type A—ECHO 13 (Garnett)

laboratory manipulation might well be the criterion of choice. It certainly makes the test for host strains easier to carry out than one which requires as a preliminary to the test an indefinite number of tissue-culture passages. This



FIG 122 Rhesus bottle cultures of polio virus Type 1 (Mahoney), Coxsackie virus Types A9 (Grigg) and B-4 (Texas 13) and ECHO virus Types 1 (Farouk) and 7 (Garnett)

leads to another problem in that we have isolated a number of Coxsackie A 9 and B strains which failed to take in infant mice unless they are first passaged in tissue culture. Obviously we are only at the beginning in the study of these agents and questions of the classification of the

Cox ECHO viruses will become resolved only as more data become available.

### PROPERTIES OF THE ECHO VIRUSES

Ultrafiltration experiments carried out with Allister Macrae have shown that ECHO viruses 1, 2 and 3 have the same size as the Coxsackie and the polio viruses. These viruses pass 38 mu graded membranes but are held back by membranes having an average pore diameter of 30 millimicrons. In experiments carried out under the same conditions Coxsackie A 9 and polio virus Type 1 had the same filtration properties (Fig. 121). Work on the size of these viruses has been continued in our laboratory using the method of ionizing radiation in collaboration with Dr. Pollard. From studies of the rate of viral inactivation produced by irradiation with high energy electrons, deuterons or alpha particles the ECHO viruses studied again proved to be similar in size to the polio viruses. It is of interest that as in the polio virus particle the infectious unit has at least twice the diameter of the complement fixing antigen as calculated from its more rapid rate of inactivation.

The growth of animal viruses in cells under agar produces areas of necrosis as first shown by Dr. Dulbecco and the morphology of these virus colonies or plaques as they are called is an aid in the characterization of these agents (Fig. 127). In chesus cultures plaques of ECHO viruses usually appear a few days later than polio virus plaques. Plaques of certain ECHO virus types are irregular in shape and often their boundaries are diffuse. In contrast to these viruses provisionally called Group A in our laboratory are the ECHO viruses in Group B. Except for some strains of Type 8 these viruses resemble polio viruses in their plaque morphology. As shown in Figure 123 in Type 17—a member of this particular group—the plaques are circular with clear centers and sharp boundaries indistinguishable from those of poliomyelitis viruses. The Group B ECHO viruses grow rapidly; the plaques being 1 cm or over in diameter 1 week after seeding. In

contrast the ECHO viruses of Group A—Type 13 in Figure 124—grow more slowly and as a rule are only  $\frac{1}{2}$  cm in diameter when measured 10 days after seeding. In natural mixed infections small amounts of polio virus particles were isolated easily and quickly by passing progeny from the larger circular plaques. Passing the progeny of the small plaques gave pure cultures of the ECHO virus (Fig. 174).

Important in the characterization of a virus is the determination of its host range. A sum-

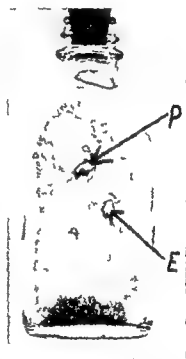


FIG. 124. Bottle culture inoculated with a stool specimen containing a mixture of virus particles. Passage of the large plaque yielded a polio virus and of the small plaque an ECHO virus. Arrows indicate sites at which agar was picked from the polio virus plaque (P) and from the ECHO plaque (E). It is advantageous to pick from a site at the boundary of a plaque where the most recent virus proliferation has occurred. Because of thermal inactivation the center of a large plaque may not contain viable virus.

TABLE 80 SUSCEPTIBILITY RATIO OF REPRESENTATIVE MONKEY SPECIES TO POLIOMYELITIS COXSACKIE AND ECHO VIRUSES\*

MONKEY	POLIO VIRUS TYPE 2†	COXSACKIE VIRUS TYPE A 9	COXSACKIE VIRUS TYPE B 1‡	ECHO VIRUS TYPE 1§	ECHO VIRUS TYPE 7
Patas ( <i>Erythrocebus patas</i> )	24	0 00000001	20	0 000000001	17
Spot nose ( <i>Cercopithecus nictitans buttikoferi</i> )	10	0 00000001		0 000000001	19
Green ( <i>Cercopithecus aethiops sabaeus</i> )	19	17		24	23
Sooty mangabey ( <i>Cercocebus fulvifrons</i> )	11	17		11	12
Olive baboon ( <i>Papio doguera</i> )	13	13		11	13
Rhesus ( <i>Macaca mulatta</i> )	10	10	10	10	10

Susceptibility Ratio =  $\frac{\text{Average plaque count in cultures of species tested}}{\text{Average plaque count in rhesus cultures}}$

† Polio virus Types 1 and 3 resemble Type 2

‡ Coxsackie viruses 2-5 resemble B 1

§ ECHO virus Types 3 4 6 9 11 13 and 14 resemble Type 1

many of work on the susceptibility of monkey kidney cells to polio Coxsackie and ECHO viruses by the plaque method is given in Table 80

Cells from a number of African species were similar to those of rhesus in their susceptibilities in contrast with those from *Erythrocebus patas* the African red grass monkey. As shown in the

first line of the table patas cells were found to be twice as susceptible as rhesus cells to polio virus and about equal to rhesus in susceptibility to the Group B ECHO viruses and Group B Coxsackie viruses. In contrast Coxsackie A 9 and the Group A ECHO viruses failed to form plaques in patas cultures a property which has proved useful in identifying new viruses. At

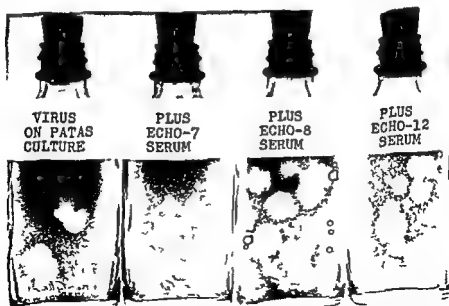


FIG. 125 Virus identification by cell susceptibility and plaque morphology. Unknown strain produced large round plaques on patas cultures as shown in bottle on the left. Neutralization occurred with ECHO 7 antiserum (second bottle from left) but not with antiserum against ECHO 8 or ECHO 12.

tually the only virus that we know which produces round plaques on rhesus and no plaques at all on patas = A9 and in every case where this has turned up we have been able to identify the virus presumptively on this property

Recently more than 200 strains of ECHO viruses were studied using the technics of plaque morphology and cell susceptibility in patas and rhesus cultures. All patas negative strains produced small irregular shaped plaques in rhesus bottles and all patas positive strains produced larger more circular plaques in cells of both species. Neutralization tests thus far indicate that the patas-positive strains were neutralized by ECHO sera against Types 7, 8 or 12, the so-called Group B ECHO viruses. A neutralization test for a newly isolated strain of Type 7 is shown in Figure 125. The control bottle shows plaques in the presence of Type 7; there are no plaques in the bottle in the presence of 8 and 12.

A classification of the ECHO viruses into Groups A and B as shown in Table 81 is supported by these studies on host cell susceptibility. Group A produce small irregular shaped plaques on rhesus but are negative on patas cultures. Group B—consisting of Types 7, 8 and 12—are positive on patas cultures except for certain strains of Type 8 which produce smaller and more irregular plaques. While the prototype strains of 2, 3, 5 and 6 have not produced typical plaques they were found to be cytopathogenic for rhesus but not for patas cells in tube cultures and are considered to be members of Group A. So far the viruses associated with aseptic meningitis cases and those actually isolated from the spinal fluid of such patients have been members of Group A.

## THE EXPERIMENTAL INFECTION OF PRIMATES WITH THE ECHO VIRUSES

To obtain a better understanding of human viral infections it has proved useful in the past to study the experimental infection in the highest laboratory primate available namely the chimpanzee. Infection of these anthropoids with ECHO viruses follows the pattern established with the other enteric viruses namely polio myelitis and Coxsackie. No apparent illness occurred among the chimpanzees but infection was readily demonstrated by the presence and persistence of virus in the throat and in the feces. It is noteworthy that Type 6 virus was found in the throat as long as it was found in the intestines and Type 4 even longer in the throat than in the intestines.

In the chimpanzees fed Type 4 virus persisted in the throat for 10 to 35 days but in the feces of these animals only from 3 to 10 days respectively. This recalls a similar observation with the Coxsackie viruses which was limited to Type B2 in which carriage of virus in the chimpanzee throat equaled that in the lower alimentary tract. Unlike polio virus which readily finds its way from the skin to the gut in the chimpanzee the ECHO viruses studied were not readily excreted after parenteral infection. In two animals infected with Type 4 virus was found briefly in the alimentary tract but this was not followed by long term carriage.

As shown in Figure 126 the chimpanzee developed neutralizing antibodies with peak titers being reached within 2 to 4 weeks. Data for 2 animals are shown in Figure 127. Antibodies persisted at their maximum level for more than 6 months and were not influenced by subsequent exposure to Type 4 virus. The anti

TABLE 81 PROVISIONAL GROUPING OF ECHO VIRUSES\*

GROUP	ECHO TYPES	GROWTH IN RHESUS CULTURES	GROWTH IN PATAS CULTURES
A†	1 3 4 6 9 11 13 14 15 16	Plaques irregular shaped small	Negative
B	7 8 12	Plaques circular large†	Positive

\*Types in italics = those which have been isolated from man. Types in parentheses = those which have been isolated from other primates. Types in bold = those which have been isolated from non-primate sources.

†Type 2, 5, 6 and the prototype strain of Type 3 (Muench) which have not yet been adapted to tissue culture also do not produce typical plaques.

‡Some Type 8 strains produce smaller and more irregular plaques.



FIG 129 Plaques of ECMO virus strain 8000 on monkey kidney monolayers (See text for details)

teric viruses for a number of different animal species

Enteric cytopathogenic monkey orphan or ECMO viruses are included among the simian viruses of Hull and his colleagues ECMO viruses—the monkey agents—have also been recovered by Cheever in Pittsburgh and by Riordan in New Haven A co-operative program among Hull's Cheevers and our laboratory is now under way in an attempt to classify the numerous agents which have been isolated In one series of 24 monkeys studied in New Haven 25 ECMO viruses have been recovered Some of these viruses were excreted for a period of weeks from the time the first isolation was made It is noteworthy that among the viruses isolated from the monkey enteric tract there have been a number of adenoviruses Our own experience in this regard was that 7 of the 25 monkey viruses isolated turned out to be adenoviruses

Some ECMO viruses have produced plaques resembling those of poliomyelitis or Coxsackie viruses described earlier others have produced plaques similar to those of the Group A ECHO

viruses Examples of ECMO plaques are illustrated in Figure 129 in which strain 8000 is shown 5 days after plating at concentrations of  $10^{-3}$  and  $10^{-4}$  At least 60 plaques are present at the higher concentration and 9 at the lower concentration This particular strain is one that is antigenically related to Hull's Sx 16 Its plaques had clear boundaries but were characterized by the presence of islets of apparently healthy cells at the periphery of the plaque These are evident as the dark spots in the plaques shown in the illustration

### SUMMARY

This paper has reviewed the developments in a field opened up by the introduction of new tissue-culture methods into virology From the time when an isolation of a new enteric virus was a rare finding often out of line with preconceived notions virologists are now at the stage of attempting to evaluate the significance of at least 20 new enteric viruses assembled together under the banner of ECHO viruses Although divided into 20 types their classification is complicated by the fact that subtypes exist which have antigenic moieties different from the prototype strains This situation is reminiscent of that which exists among the influenza viruses

Although newly recognized the ECHO viruses appear to be among the most common and widespread parasites of man Yet the only illness with which any of these viruses have been associated is aseptic meningitis and thus they often confuse the picture of nonparalytic poliomyelitis Among the ECHO viruses only members of Group A have been isolated from the cerebrospinal fluid or have been otherwise associated with aseptic meningitis It may well be that certain ECHO types are not pathogenic for the human species and that their isolation from man has no more significance than the isolation of saprophytic bacteria or certain members of the Coxsackie group which have not yet found their disease And yet because we know so little about the factors which might lead a virus toward an increase in virulence and the production of serious illness the continued study of the ECHO viruses would seem to be a highly desirable pursuit

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# Outbreaks of Aseptic Meningitis Caused by Type 6 ECHO Virus

DR DAVID T KARZON

An outbreak of aseptic meningitis caused by ECHO-6 in western New York State during the summer of 1955 was studied. Aseptic meningitis associated with ECHO-6 virus has also been observed in northeastern United States by Kabrick, Melendes and Enders and by Davis and Melnick in 1954 and 1955. In the present study 156 hospitalized aseptic meningitis patients and 485 household associates were examined (Table 87). ECHO-6 virus was recovered in monolayer monkey kidney tissue culture from stools, throat swabs and spinal fluids of 68 per cent of the patients as seen in the column on the right while polio virus was recovered from 4 per cent and other cytopathogenic agents from 6 per cent. ECHO-6 was recovered from the spinal fluid of 8 patients. Neutralizing antibody to ECHO-6 appeared by the fifth day of illness and persisted for at least 7 months. ECHO-6 was also isolated from 50 per cent of the household associates with minor illness and in 16 per cent of those without illness.

The clinical findings (Table 83) were reviewed in 107 patients determined to have ECHO-6 aseptic meningitis by virus isolation. Fever, headache and malaise occurred in almost all cases. Pain, stiffness and muscle spasm especially in the back, the neck and the hamstring muscles were usually present. Approximately one half the cases had abdominal or chest pain. Gastrointestinal complaints, vomiting, anorexia

or nausea occurred in approximately 90 per cent of the cases. Constipation and diarrhea occurred in less than 15 per cent. Photophobia, sore throat and pharyngeal infection were frequent. Changes in sensorium such as delirium and dis-

TABLE 83. CLINICAL FINDINGS IN 107 CASES OF ASEPTIC MENINGITIS DUE TO ECHO VIRUS TYPE 6

General	
Fever	106
Headache	100
Malaise and fatigue	31
Pain	
Muscle pain or spasm	73
Abdominal	43
Chest	17
Gastrointestinal	
Vomiting	81
Nausea or anorexia	11
Constipation	14
Diarrhea	13
Mucous membranes	
Photophobia	24
Sore throat	16
Cough	6
Central nervous system	
Irritability and dizziness	7
Delirium and disorientation	3
Neuromuscular	
Stiff neck and back	99
Weakness	32
Absent superficial reflexes	16
Absent deep tendon reflexes	18

TABLE 87. VIRUS ISOLATIONS FROM 156 CASES OF ASEPTIC MENINGITIS IN ERIE COUNTY, NEW YORK, IN 1955

VIRUS	STOOL (127)		THROAT (115)		CSF (108)		TOTAL ISOLATIONS (136)		
	NO.	POS. PER CENT	NO.	POS. PER CENT	NO.	POS. PER CENT	NO.	POS. PER CENT	
ECHO 6	78	61%	48	42%	8	8%	92	68%	
Polio virus	6	5%	4	3%	0	0	6	4%	
Other enteric	11	4%	1	1%	3	3%	8	6%	
Total	111	70%	53	46%	11	11%	106	78%	

## SUMMARY

The presently reported outbreak in western New York State in 1955 offers the following evidence that ECHO-6 is a cause of aseptic meningitis (1) a high recovery rate of ECHO 6 from aseptic meningitis cases (2) the demonstration of a specific neutralizing antibody response (3) the isolation of ECHO-6 from a parenteral site the cerebrospinal fluid and (4) the demonstration of an epidemiologic association of ECHO 6 with aseptic meningitis cases and their households in a defined outbreak

The ECHO-6 virus group has been shown to be highly heterogeneous antigenically. A mechanism of serologically directed variation of strain pattern has been proposed to account for this observation

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# *Benign Aseptic Meningitis*

DR HERBERT A WENNER

In 1935 Wallgren described the clinical picture of acute aseptic meningitis. Since that time a variety of filtrable viruses have been found in association with febrile illnesses involving the central nervous system. Polio myelitis, Coxsackie lymphocytic choriomeningitis and mumps virus and members of leptospiral species have been listed as major causes of illnesses resembling those described by Wallgren. Now as Dr Melnick and Dr Harzon have indicated we have the ECHO group added to the list.

Our experience with ECHO virus Type 4 began in July 1955. At that time an illness resembling nonparalytic polio myelitis occurred in Marshall County, Iowa. Between mid July and August 9, 1955, an estimated 700 patients were seen by practicing physicians in the area.

A preliminary survey of the community during the early days of August by the team from the United States Public Health Service indicated that the current illness was widely distributed in the urban population and in regional small towns.

The onset of illness was generally abrupt. Fever present in all cases was characteristically benign, ranging from 99° to 103° F. The febrile period lasted 3 to 5 days. Headache was a frequent complaint; painful extension of the neck was also a frequent symptom. Myalgia involving the lower extremities was encountered in more than half the patients (51%). The acute phase of illness lasted 4 to 6 days followed by general malaise for several days thereafter.

The physical findings consisted of moderate redness of the pharynx, stiffness of the neck and the back and only exceptionally muscle weakness. Gross evidence of muscular paralysis was not observed. The deep tendon and the superficial reflexes were normal. Muscular weakness of a transient and minor nature was observed in leg muscles in 2 patients.

An increase in the spinal fluid cells was encountered in 16 hospitalized patients. The total cell count ranged between 16 and 900 cells per

cubic mm, the average value being 200. Cells were predominantly lymphocytes. Cerebrospinal fluid sugar and protein values generally within the normal range. The highest protein value encountered was 67 mg per 100 cc.

In addition to the patients presenting signs and symptoms of aseptic meningitis, a large number of persons were encountered with minor illnesses. Characteristic features of the minor illnesses were low grade fever, headache, sore throat and irritability—particularly in children. Evidence of meningeal irritation was not encountered in these individuals. Unfortunately it was not possible to obtain cerebrospinal fluid from the individuals with minor illnesses.

The epidemic ran its course within a period of about a month. The peak incidence occurred between July 20 and August 13, 1955, based on reported and surveyed cases indicating an average attack rate of 19 per cent. In the urban area of Marshalltown the highest attack rates (76%) were encountered in areas of low economic status, along with a trend for occurrence of secondary cases in larger sized families. Assuming that the population group chosen for survey represented a random sample, interpolation of the average rate (16%) for Marshalltown indicated that as many as 3,700 individuals may have experienced infection with ECHO Type 4 virus during the course of the epidemic.

The age specific attack rates for frank aseptic meningitis (Table 86) were highest in children (31%). Attack rates for adults were lower, ranging between 14 and 19 per cent. Multiple cases occurred in family associations. The larger the size of the family group, the greater was the likelihood of encountering aseptic meningitis or minor illness. The data indicate that the household as a group experienced infection with virus or was spared. In many instances infection was an all-or-none phenomenon. When secondary cases occurred within a family group they usually appeared

TABLE 86 COMPARISON OF AGE SPECIFIC ATTACK RATES BY TYPE OF ILLNESS  
(SURVEY CASES ONLY) MARSHALL COUNTY IOWA SUMMER 1955

AGE IN YEARS	SURVEY POPULATION	CASES WITH MENINGEAL SYMPTOMS		ASSOCIATED MINOR ILLNESS		TOTAL	
		NUMBER	RATE PER 100	NUMBER	RATE PER 100	NUMBER	RATE PER 100
0-14	214	36	16.8	30	14.0	66	30.8
15-20	93	10	10.8	8	8.6	18	19.4
30-44	135	15	11.1	5	3.7	20	14.8
45-59	96	4	4.2	9	9.4	13	13.6
60 & over	65	2	3.1	1	1.5	3	4.6
Total	603	67	10.7	53	8.4	120	19.1

within 5 days after onset of the index case. Whether this indicates a relatively short incubation period or whether most of these cases were *co* primaries could not be accurately determined.

Cytopathogenic agents were recovered in renal cell cultures from 23 of 57 individuals studied (Table 87). Twenty-one agents were detected in 57 fecal specimens. Two of 7 individuals studied yielded an agent in throat washing. The single spinal fluid tested was negative in tissue culture.

Nineteen of the 23 agents were specifically neutralized by ECHO Type 4 antisera. None of the agents was neutralized by poliomyelitis or Coxsackie antisera. Each of the 4 remaining positive fluids was neutralized by a mixture of ECHO Type 4 and poliomyelitis antisera. Three specimens yielded in addition to ECHO Type 4 poliomyelitis virus Type 1 and one yielded polio virus Type 3.

Serologic evidence that ECHO virus Type 4 was related to illness was obtained with 15 paired sera from sick individuals and their family associates. A fourfold or greater rise in serum neutralizing antibody was observed in 14 paired sera. Three additional sera contained neutralizing antibody at the time when the first serum was obtained and a rise in titer was not observed. The remaining 8 individuals did not have specific antibodies in early or later bleedings.

If the data are examined according to history of illness (Table 88) the following apply. Ten of 13 individuals with aseptic meningitis had an increase of fourfold or greater in homotypic antibodies. Of the 3 remaining individuals 1 had pre-existing antibodies without subsequent change; the remaining 2 did not have nor did they develop specific antibodies beyond the 1:4 or 1:8 serum dilution tested.

TABLE 87 FREQUENCY OF VIRUS RECOVERY FROM 57 INDIVIDUALS BY CLINICAL STATUS  
MARSHALLTOWN IOWA EPIDEMIC 1955

CLINICAL STATUS	VIRUS RECOVERY (ECHO 4)		
	NUMBER INDIVIDUALS	NUMBER POSITIVE	PER CENT POSITIVE
Aseptic Meningitis	31	17	55
Minor Illness	15	4	27
No Illness (Familial Contacts)	11	2	18
Total	57	23*	40

\* Virus recovered from the following: 2 patients and from feces of two others. One had mixed infection with ECHO 4 and Polio 1; 1 with ECHO 4 and Polio 3.

TABLE 88 NEUTRALIZING-ANTIBODY RESPONSE TO ECHO 4 AMONG 25 INDIVIDUALS BY CLINICAL STATUS MARSHALLTOWN IOWA EPIDEMIC 1955

CLINICAL STATUS	NUMBER INDIVIDUALS	RISE 4 X OR GREATER	NO SIGNIFICANT CHANGE IN TITER	NO ANTIBODY
Aseptic Meningitis	13	10	1	2
Minor Illness	8	2	1	5
No Illness (Familial Contacts)	4	2	1	1
Total	25	14	3	8

Total of 14 18

The occurrence of multiple infections in affected households was observed. Two families were studied and are presented here in respect to serologic conversion. One of these families is recorded in Table 89. The illness in this family extended to involve 5 of the 7 members during a brief period the onset of illness in 4 members occurred within a 4-day interval. Four children had aseptic meningitis. 1 adult experienced a minor illness. ECHO virus Type 4 was recovered from the feces of 2 children with aseptic meningitis; the virus was not detected in the remaining 2 siblings. Stools were not obtained from the children until 15 to 25 days after onset of illness. A rise in serum neutralizing antibodies occurred in sera obtained from the 3 individuals experiencing illness in the household. The second family represents the

occurrence of inapparent infection in the family unit. One member of the family became ill with signs and symptoms of aseptic meningitis. None of the other 6 members developed a recognizable illness. ECHO virus Type 4 was found in the feces of 1 of the adults but not in the 3 teenagers. The virus carrier had specific antibodies without subsequent rise whereas antibody rises were observed in 3 other individuals in the family.

The epidemic of benign aseptic meningitis occurring in Marshall County Iowa during the summer of 1955 was associated closely with ECHO virus Type 4. The data do not provide unequivocal evidence that ECHO virus Type 4 was the virus responsible for illness; the recovery of this virus from patients and the subsequent rises in type specific antibodies strongly suggest

TABLE 89 OCCURRENCE OF INFECTION IN A HOUSEHOLD—THE OM FAMILY—MARSHALLTOWN IOWA EPIDEMIC 1955

FAMILY MEMBERS	AGE (YEARS)	ILLNESS HISTORY	DATE OF ONSET	VIRUS ISOLATION (STOOL)	ECHO 4 SERUM NEUTRALIZING ANTIBODY TITER (DATE)
OM Sr	76	Minor Illness	8/6	No Specimen	<4
IM	25	No Illness	—	No Specimen	>128
JM	8	Aseptic Meningitis	7/28	8/12 ECHO 4	No Specimen
OM Jr	6	Aseptic Meningitis	7/26	8/18 Negative	8/10 10
JAM	5	Aseptic Meningitis	7/24	8/18 Negative	8/10 82
RM	3	No Illness	—	No Specimen	8/10 16
MM	2	Aseptic Meningitis	7/24	8/18 ECHO 4	10/14 >128
				8/18 Polio-I	No Specimen
					No Specimen

Titer expressed as reciprocal of titer serum dilution

TABLE 93 COXSACKIE VIRUSES NEW TYPES

GROUP	COLLECTION NUMBER	CYTOPATHOGENICITY			OTHER CHARACTERISTICS
		MONKEY KIDNEY CELLS	HELa CELLS	UTERINE PLASMA CLOT	
A	5536	—	+	—	Isolated in Louisiana † Serologic cross reactivity with serum of strain No 55166 Not identical
A	55161	—	+	+	Isolated in California ‡ Induces paralysis in some 10 to 12 Gm mice‡
A	55166	—	+	—	Isolated in New York State on HeLa cells Serologic cross reactivity with serum of strain No 5536 Not identical
A	55222	+	—	—**	Isolated in Italy on monkey kidney cells aseptic meningitis epidemic§  Cross neutralization with serum prepared with ECHO Type 9 representative strain*
A	5630	—	—	—	Isolated in New York State in suckling mice
B	5592	+	—	—	Representative strain of ECHO 10 (Sabin) <sup>Δ</sup> Grows in eggs

Not cytopathogenic when tested on HeLa cells

Other strains of the type cytopathogenic on human uterine cells Titer 1:

† Kilbourne II D and Goldfield M Am J Med 21 1:5 1956

‡ Dalldorf G The new cytopathogenicity of Group A Coxsackie virus J Exp Med 106 69 1957

§ Azhetu I and Fl A Riv Ist Roterap Ital 31 265 1956

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<sup>Δ</sup> bh w M N w York State Dept of Health Bull of Laboratories and Research Ann 1 Rep rt 1956

Table 92 lists the 5 viruses in Group B and they are readily handled in tissue-culture testing although there are differences. Generally monkey kidney cells are very satisfactory in picking up the B's.

Table 93 shows the additional types that have not been assigned type numbers as yet. Type 55222 which we first received from Italy and which has been mentioned previously as ECHO 9 is quite similar to A 9 in its behavior in tissue culture. Of the others some of them are rather similar to the higher types of A that appeared in Table 91.

Our present practice for isolation of infectious agents from the feces of patients makes use of both tissue culture and suckling mice. The tissue cultures include trypsinized monkey kidney cells, HeLa cells and human uterine tissue in

plasma clot. We hope soon to include human amnion cell culture as well. We have found that the spectrum of cytopathogenicity obtained frequently gives us a clue to the sera for use in our preliminary screening test. The suckling mice are 1 day old or less since some strains are not readily isolated even in 2-day-old animals. Inoculations are made by 3 routes. First passage is also made in 1 day-old mice using both brain and muscle by the same 3 routes—intracerebral, subcutaneous in the interscapular area and intraperitoneal.

For some strains usually Group B repeated passage is necessary before all mice are uniformly infected and a neutralization test is possible. For these passages with Group B strains we use the subcutaneous route and both brain and muscle tissue. For Group A the intraperi-

TABLE 94. The Coxsackie Viruses—1957  
VIRUS STRAINS ISOLATED FROM 308 SEWAGE SPECIMENS INOCULATED INTO NEWBORN MICE  
AND MONKEY KIDNEY OR HELa CELL CULTURES

AGENT	SERIES A			SERIES B		
	MICE ONLY	HELa CELLS ONLY (208 SPECIMENS)	SIMULTANEOUSLY IN MICE AND HELa CELLS	MICE ONLY	MONKEY KIDNEY TISSUE ONLY (100 SPECIMENS)	SIMULTANEOUSLY IN MICE AND MONKEY KIDNEY TISSUE
Coxsackie A	63	0	0	41	0	0
Coxsackie B	6	3	1	9	10	10
Poliovirus	0	64	0	0	33	0
Orphans	0	3	0	0	2	0
All Agents	69	70	1	50	45	10

tonal route is used with muscle suspension since for many of the newer types the brain suspensions contains very little virus.

From our 1956 specimens in only one case was a Coxsackie virus isolated in tissue culture and not in suckling mice. This was a Group A Type 9. However, several Group B Type 3 strains required what was equivalent to blind passage though guided by histologic lesions and might have been missed. One virus, Group B Type 3, was isolated by this method in suckling mice but was not recovered in tissue culture.

Occasionally a Coxsackie virus has proved to be isolated more readily in tissue culture than in mice. It is important to realize that many Coxsackie viruses including a number of the more interesting and possibly more important types may be isolated only in mice. They have not been cytopathogenic for any of the cultured cells so far tested by us. In these days when for practical reasons many laboratories rely largely on tissue culture it is necessary to remember the present limitations of this method. I recently tabulated the results of a number of large studies in which the viruses were identified and typed by experienced workers. Of a thousand strains approximately one (fourth) fell in Group B. These presumably would have been cytopathogenic on monkey cells and many but not all on HeLa cells. The other 730 fell in Group A and 95 per cent of them could not be cultivated on tissue culture with our present techniques.

This is a serious problem and deserves emphasis. My associate, Dr. Sally Kelly, who is interested in the sanitary aspects of the enteric viruses has been looking for them in sewage

Each year's experience has emphasized the differences between the results of the mouse and the tissue-culture tests. In a recent summary she reported the results of testing 308 sewage samples that yielded 245 enteric viruses (Table 94). Of these 119 were isolated only in suckling mice, 115 only in tissue culture and 11 by both methods.

Similar results have been reported from Dr. Rhodes's laboratories where the specimens were tested in mice, tissue culture and monkeys. At first suspended cell cultures of monkey testis were used but later the specimens were retested on monolayers of monkey kidney cells. The final results as reported by Dr. Beale and his colleagues illustrate the importance of the technique.

NUMBER OF STRAINS OF COXSACKIE AND POLIO VIRUSES ISOLATED

	IN MICE TESTS		IN MONKEY KIDNEY MONKEYS	
Coxsackie viruses	18	9	13	0
Polio viruses	0	3	3	5

Only half of the Coxsackie viruses could be isolated on monkey testis and only two thirds on monkey kidney cell cultures. The kidney cells were more susceptible to the Group B viruses. The comparative results in monkeys and tissue culture are equally interesting. 2 polio viruses were isolated in monkeys that were



TABLE 93 COXSACKIE VIRUSES NEW TYPES

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‡ Dalldorf G The n ropathogeni ty of Group A Co sackie viruses J E pe M d 106 69 1957

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Our present practice for isolation of infectious agents from the feces of patients makes use of both tissue culture and suckling mice. The tissue cultures include trypsinized monkey kidney cells, HeLa cells and human uterine tissue in

plasma clot. We hope soon to include human amnion cell culture as well. We have found that the spectrum of cytopathogenicity obtained frequently gives us a clue to the sera for use in our preliminary screening test. The suckling mice are 1 day old or less since some strains are not readily isolated even in 2-day-old animals. Inoculations are made by 3 routes. First passage is also made in 1 day old mice using both brain and muscle by the same 3 routes—intracerebral, subcutaneous in the interscapular area and intra-peritoneal.

For some strains usually Group B repeated passage is necessary before all mice are uniformly infected and a neutralization test is possible. For these passages with Group B strains we use the subcutaneous route and both brain and muscle tissue. For Group A the intraperi-

TABLE 94 VIRUS STRAINS ISOLATED FROM 308 SEX-AGE SPECIMENS INOCULATED INTO NEW-BORN MICE AND MONKEY KIDNEY OR HELA CELL CULTURES

AGENT	SERIES A			SERIES B		
	MICE ONLY	HELA CELLS ONLY (208 SPECIMENS)	SIMULTANEOUSLY IN MICE AND HELA CELLS	MICE ONLY	MONKEY KIDNEY TISSUE ONLY (100 SPECIMENS)	SIMULTANEOUSLY IN MICE AND MONKEY KIDNEY TISSUE
Coxsackie A	63	0	0	41	0	0
Coxsackie B	6	3	1	9	10	10
Polyomyelitis	0	64	0	0	33	0
Orphans	0	3	0	0	2	0
All Agents	69	70	1	50	45	10

toneal route is used with muscle suspension since for many of the newer types the brain suspension contains very little virus.

From our 1956 specimens in only one case was a Coxsackie virus isolated in tissue culture and not in suckling mice. This was a Group A Type 9. However, several Group B Type 3 strains required what was equivalent to blind passage though guided by histologic lesions and might have been missed. One virus Group B Type 3 was isolated by this method in suckling mice but was not recovered in tissue culture.

Occasionally a Coxsackie virus has proved to be isolated more readily in tissue culture than in mice. It is important to realize that many Coxsackie viruses including a number of the more interesting and possibly more important types may be isolated only in mice. They have not been cytopathogenic for any of the cultured cells so far tested by us. In these days when for practical reasons many laboratories rely largely on tissue culture it is necessary to remember the present limitations of that method. I recently tabulated the results of a number of large studies in which the viruses were identified and typed by experienced workers. Of a thousand strains approximately one fourth fell in Group II. These presumably would have been cytopathogenic on monkey cells and man but not all on HeLa cells. The other 730 fell in Group A and 93 per cent of them could not be cultivated on tissue culture with our present techniques.

This is a serious problem and deserves emphasis. My associate Dr. Sally Kelly who is interested in the sanitary aspects of the enteric viruses has been looking for them in sewage

. Each year's experience has emphasized the differences between the results of the mouse and the tissue-culture tests. In a recent summary she reported the results of testing 309 sewage samples that yielded 245 enteric viruses (Table 94). Of these 119 were isolated only in suckling mice, 115 only in tissue culture and 11 by both methods.

Similar results have been reported from Dr. Rhodes's laboratories where the specimens were tested in mice, tissue culture and monkeys. At first suspended cell cultures of monkey testis were used but later the specimens were retested on monolayers of monkey kidney cells. The final results as reported by Dr. Beale and his colleagues illustrate the importance of the technique.

NUMBER OF STRAINS OF COXSACKIE AND POLIO VIRUSES ISOLATED

	IN MICE		IN MONKEY TESTIS	IN MONKEY KIDNEY	IN MONKEYS
Coxsackie viruses	18	9	13	■	■
Polio viruses	0	3	3	5	5

Only half of the Coxsackie viruses could be isolated on monkey testis and only two thirds on monkey kidney cell cultures. The kidney cells were more susceptible to the Group B viruses. The comparative results in monkeys and tissue culture are equally interesting. 2 polio viruses were isolated in monkeys that were

not isolated in tissue culture. One of the misses was shown by the mouse tests to be a mixture of a Group B Coxsackie and a polio virus. These two are known to interfere in tissue culture and the failure to isolate the polio virus in tissue culture may have been due to interference. The monkey being insusceptible to the Group B virus responded to the polio virus that was suppressed in the kidney cells.

The other failure was one in which a Group A virus was isolated in mice from an inoculum that caused poliomyelitis lesions in a monkey. The monkey virus was not typed. It is conceivable as will be showed later that the lesions in the monkey were due to the Group A virus.

Therefore the failure to find a virus must be interpreted in terms of the technics that were used. This qualification applies to mouse tests as well as to the methods and the cells used in tissue culture work. For example, Johnsson compared the results of mouse tests in which 2 routes of inoculation were used (see bottom of page).

One may see readily what strikingly different results the two technics got and how both technics were required to find most of the viruses there. I have not tabulated our own experience of this kind but it would be similar. We would not do it precisely as Dr. Johnsson has but the principle is right and we certainly agree that the sample he has is representative of our experience.

In his experience the intracerebral route was superior for Group B viruses and the intraperitoneal or subcutaneous for Group A. I have the impression that we would prefer the subcutaneous route for Group B viruses if we were reduced to a single test.

To summarize, current tissue-culture technics are inadequate for the isolation of many Group A viruses. They are satisfactory in isolating Group B viruses. Each method has its limitations and a thorough examination requires the use of several technics. I suspect that the thor-

ough investigation of a few patients may be more instructive than testing many by a single method.

Our primary interest in the Coxsackie viruses has always been their pathogenicity for man in the nature of the diseases they cause. In the beginning there were some who doubted that these viruses were human pathogens and this seemed to be a reasonable position because in fact there were no diseases with which to associate them. If there were no diseases caused by Coxsackie viruses the viruses must be nonpathogenic. However, by the time we met in 1951 suggestive evidence of pathogenicity had begun to accumulate. Dr. Curnen discussed the subject in Copenhagen. It was he who first suggested that Coxsackie viruses might cause epidemic pleurodynia or Bornholm disease. In 1951 the evidence was incomplete and Curnen was also uncertain whether the association held for particular types or not.

We need not go into that subject in detail. An important extension to our knowledge of epidemic pleurodynia has come from the Swedish studies where different clinical patterns were found in the young and in adults—aseptic meningitis in the children and pleurodynia in their parents. A second significant extension of our knowledge has come from the opposite end of the globe from South Africa where Dr. Gear and his associates discovered the nature of Group B infections in the newborn. We now know of 3 different manifestations of Group B infections: frequently fatal myocarditis in the newborn, aseptic meningitis in young children and epidemic pleurodynia in adults.

In those years when we were interested in the Group B viruses we felt that they were the more important but our own experience in the past 2 years has changed our view a little in suggesting that the Group A viruses may have significance beyond what we once thought.

Originally they had been isolated from the

EFFECT OF THE ROUTE OF INOCULATION ON THE ISOLATION OF COXSACKIE VIRUSES\*

GROUP	NO ISOLATED BY ALL METHODS	NO ISOLATED BY IC ONLY	NO ISOLATED ONLY BY SC OR IP	NO ISOLATED BY EITHER METHOD
A	85	5	27	51
B	67	23	12	37

\* John and A. Virological and Clinical Study of Infections with Coxsackie Viruses. Stockholm: Uddéalla Bokhandeln, 1955.

feces of 2 boys with paralytic poliomyelitis. The more that was learned about them the more they resembled the polio viruses. But there was one great discrepancy. The lesions in experimental animals in suckling mice were exclusively of the striated muscles. I had never seen a human disease that resembled the one in mice and the few efforts we made to find such a disease or lesions were disappointing. We know nothing of course of the lesions in man because we had no fatalities to examine.

Now and then we found a clue that suggested that certain of the Group A viruses might be neurotropic. A few minor lesions had been seen in the spinal cords of newborn guinea pigs and occasionally an adult mouse inoculated intracerebrally with a Group A strain became paralyzed.

This has been noted recently with 3 strains representing 3 different Group A types. It encouraged us to attempt to adapt these strains to adult mice. One of the 3 the representative strain of Group A Type 14 has now been established in adult mice and while the adaptation is incomplete and does not suggest the emergence of a mutant but rather the behavior of Group B strains that have been adapted to adult mice nevertheless it is possible to cause paralysis in three fourths of the animals. The virus causes myositis in infant mice and poliomyelitis in adult mice. Animals of intermediate ages may show both lesions so far however only traces of the second have been found. The virus is neutralized only by homologous antiserum in both infant and adult mice and is not neutralized by antiserum for mouse encephalomyelitis encephalomyocarditis or the 3 polio viruses.

This strain also causes lesions in monkeys. The disease in monkeys has been a mild one thus far without fatalities or frank paralysis but the changes in the neuraxis are unmistakable. There seems to be no reason to doubt that this Coxsackie A 14 strain causes nonparalytic poliomyelitis in cynomolgus monkeys and I suspect that it may do the same in man that the human disease is also a mild form of poliomyelitis. This of course remains to be proved but judging from our experience with classical poliomyelitis it would seem to be a reasonable assumption.

Russian workers have isolated a virus from paralytic and fatal cases of poliomyelitis which

they call Type 4 polio virus. Professor Chumakov gave this strain to one of my colleagues and we have had no difficulty confirming his observations. The virus causes necrosis of striated muscles in suckling mice. It is not pathogenic for adult mice. In other words it has the characteristics of a Group A Coxsackie virus. In monkeys it causes a disease resembling paralytic poliomyelitis. The lesions are similar to those caused by the A 14 virus and by the 3 polio viruses. We found Chumakov's virus to be an old acquaintance a well known Group A Coxsackie virus Type 7. We have tested 2 of our own A 7 viruses originally isolated in 1949 but reisolated from the original fecal specimens for these tests and find that both of them cause lesions in monkeys. The general practice has been to discount the significance of Coxsackie viruses isolated from cases of paralytic poliomyelitis and some of my colleagues suspect that a polio virus may have been present as well as the A 7 in the Russian patients. However in view of the monkey pathogenicity this does seem to be a little arbitrary and we should seriously consider the possibility that the Coxsackie virus was responsible.

Something more should be said about the lesions which I have mentioned and described by the term poliomyelitis. It may be that they can be distinguished from those in monkeys inoculated with the 3 established types of polio virus. Indeed I think this is not unlikely. My own observations suggest that there are differences. But in their essential characteristics all are similar. There is clear-cut evidence of degeneration and necrosis of motor neurones in the spinal cord and the medulla; there is reaction characterized by perivascular infiltrations and glia cell proliferation in the stroma. It is a poliomyelitis. We need not be concerned to call it that if we remember that the lesion of poliomyelitis is not specific of infections caused by the polio viruses.

Whatever the final answers to these questions may be there is now evidence that several of the Group A viruses can cause disease in adult mice and monkeys and that the disease resembles poliomyelitis. There are other reasons to suspect that we may have underestimate the Group A viruses. Halbourne and Golfield have carefully studied patients with acute infections of the central nervous system and came to that

conclusion. Johnsson examined 23 of his patients by means of electro-encephalographs and found that 9 had abnormal patterns. Three of the 9 were A 7 infections. This clearly means parenchymal damage. Finally I may say that the disease in animals agrees very well with the weakness and the transient paralysis that we frequently have seen in association with Group A infections. Interestingly one of Kilbourne's patients from whom an A 7 virus was isolated also had transient paralysis. Two more severely ill patients were infected with A 9.

It should be emphasized however that the clinical data we have as a whole indicate that Group A infections are relatively benign diseases despite their tendency to mimic poliomyelitis. We have seen no fatalities nor do I know of a proved case with permanent paralysis. Whatever their potentialities these diseases with the possible exception of the Russian cases so far have been benign. They have been avirulent forms of poliomyelitis.

One characteristic of the Coxsackie viruses that has long interested us has been the annual change in the predominant types. I mentioned this in 1951 but since then we have seen more striking examples. Indeed the year we met—1951—proved to be remarkable for the prevalence of B 3 strains. Outbreaks were identified in many parts of the world. In 1948 B 1 had been the epidemic type. A world wide prevalence of B 3 such as in 1951 has not occurred so far as we know but the experience in 1955 and 1956 when epidemics of aseptic meningitis due to a particular Group A virus occurred in most European countries and Canada is equally striking.

It is possible that we may see the emergence of new varieties as in influenza but I suspect that we are witnessing a somewhat different phenomenon. When poliomyelitis first appeared in epidemic form not so long ago some considered it to be new despite the evidence that it had occurred in ancient times. The newness was the newness of epidemic poliomyelitis. Whatever the environmental changes that have been responsible for the increase in poliomyelitis during the recent past it is not likely that they may have changed the relationship between man and these other enteric viruses as well and that they too are now for the first time emerging as epidemic diseases? Dr Gear has predicted this

as standards of living are improved so will the incidence of paralytic poliomyelitis increase. There is every reason to believe that the same holds true for the Coxsackie viruses.

Unfortunately we have little morbidity data. Windorf by means of personal inquiry documented the prevalence of epidemic pleurodynia in Germany and learned that it is increasingly common and the outbreaks more numerous and larger. Last summer California had the biggest epidemic in the history of that state. It should be kept in mind that the geographic distributions of epidemic poliomyelitis and pleurodynia are very similar. The earliest large epidemics occurred in Scandinavia. Indeed the disease was reportable in Denmark for a number of years. It is my impression that we see more of these diseases in New York than we did although one cannot count the cases that might not have been brought to our attention years ago. Possibly the newness of the disease noted in Europe during the past 2 years and I am thinking principally of the reports that the rash was unique represents another emergence of an old disease in epidemic form. Scattered sporadic cases attract little attention but epidemics always impress us. Our reactions to epidemics are conditioned by the adventures of our forebears. These are topics that may interest future Congresses.

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## *Epidemics of Aseptic Meningitis Due to Coxsackie B-5 Virus*

DR MICHAEL L. FURCOLOW

The Coxsackie viruses which have been reviewed so well by Dr Dalldorf have been responsible for many types of illness including epidemics of aseptic meningitis. However outbreaks of this disease due to Coxsackie Type B5 have not been described in the literature. I shall give a preliminary report on one major epidemic and several small ones due to Coxsackie P5 which occurred in the central United States.

Figure 137 illustrates the frequency of isolations of Coxsackie B5 virus using monkey kidney tissue cultures from cases of suspected poliomyelitis during the summer of 1956. The denominator of the fractions shown represents the number of stools tested from each state and the numerator represents the number of isolations of B5. The dots represent single isolations. Note that the Coxsackie B5 virus isolations were limited essentially to the 4 northern states although in Oklahoma the number of cases studied was small. In Arkansas no isolations were made from 104 individuals. The greatest number of isolations came from Iowa where Coxsackie B5 infection occurred in epidemic proportions in several areas of the state. The virus was recovered from 144 individuals, 73 of whom were from one county—Cerro Gordo County—where an extensive investigation was carried on. The isolations from Nebraska were mainly from the largest city. In Missouri and Kansas the isolations were scattered. Only 3 strains of polio virus were recovered from Iowa while poliomyelitis virus was recovered frequently from the patients in the other states.

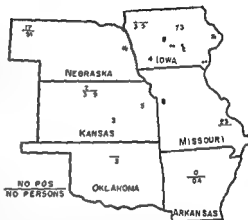


FIG 132 Frequency and distribution of Coxsackie B5 isolations from stools collected in 6 midwestern states in 1956. The numerator represents the number of individuals with isolations and the denominator the total number of individuals studied.

suspected due to reports of increasing incidence of nonparalytic poliomyelitis during the second and third weeks of July.

The study in Cerro Gordo County was designed to (1) define the clinical characteristics of the disease and (2) estimate the incidence of the disease and characterize the epidemic. The clinical characteristics of the disease were defined by studies of reported cases and their families. Any case reported by a physician was considered a reported case. These included both hospitalized and nonhospitalized cases.

The incidence of the disease was estimated by a random household survey encompassing both the city and the rural areas. More than 1500 persons were interviewed using standard questionnaire forms. A survey was conducted on August 16 and a second survey was made 8 weeks later to determine whether cases had continued to occur following the decline of reported cases.

### DESCRIPTION OF THE MAJOR EPIDEMIC

The epidemic in Cerro Gordo County, Iowa, was first noticed in Mason City, the county seat. Mason City has a population of 33,000 and is located in the central part of the county which contains a population of 46,053. The occurrence of an epidemic illness in this county was first



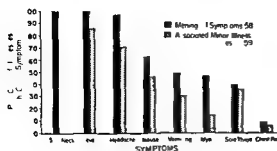


FIG 133 Distribution of symptoms by type of illness (reported cases)

### CLINICAL PICTURE

The spectrum of illness observed in the epidemic was found to be extremely broad. Laboratory evidence of infection was observed in asymptomatic family contacts as well as in those with minor illnesses and aseptic meningitis. In general the illness was characterized by fever, headache, stiff neck, nausea and vomiting, myalgia and sore throat. Figure 133 illustrates the frequency of different symptoms among both the cases with meningeal irritation and those with minor illnesses. Stiff neck as well as headache and fever were present in all those cases classified as aseptic meningitis. The symptoms among the group classified as associated minor illnesses resembled those seen in the cases with clinical evidence of meningeal irritation.

The illness lasted from 2 to 22 days with a median of 9 days. The temperature ranged as high as 105 F with a median of 102°. The physical findings were notable for their absence although the patients usually appeared to be acutely ill and nuchal rigidity and injection of the pharynx were commonly seen. Although transient muscle weakness was observed no permanent paralysis was noted. The clinical laboratory findings were negative except for spinal fluid pleocytosis. The spinal fluid cell count ranged from 0 in one case to 2,400 per cu mm averaging 570. Early in the disease polymorphonuclears tended to predominate while during the later stage mononuclears were more numerous. Total protein usually was not elevated above normal. It is of interest that spinal fluid pleocytosis was found in 7 patients in whom no signs of meningeal irritation could be elicited.

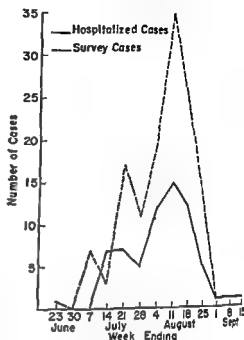


FIG 134 Comparison of hospitalized survey cases by week of onset

## EPIDEMIOLOGY

### EPIDEMIC CURVE

Figure 134 shows the distribution of cases by week of onset for the hospitalized and the survey cases in Cerro Gordo County. It is evident that the peak incidence for both groups occurred during the week of August 11 with a majority of the cases appearing during the first 3 weeks of August. Only 3 cases were reported after the last week of August.

### ATTACK RATES

Table 95 shows the illness rates observed in a random survey of 1,542 persons in Cerro Gordo County. A total of 8 per cent had a history of

TABLE 95 INCIDENCE AND TYPE OF ILLNESS OBSERVED IN A SURVEY GROUP

TYPE OF ILLNESS	NUMBER	PER CENT
Aseptic meningitis	27	1.7
Minor illness	95	6.2
No illness	1,420	91.1
Total	1,542	100.0

## Epidemics of Aseptic Meningitis Due to Coxsackie B 5 Virus

illness 2 per cent of which could be classified as aseptic meningitis and slightly over 6 per cent as minor illness. Based on the incidence rate observed in this survey it was estimated that over 3 000 cases had occurred during the course of the epidemic. A resurvey of the same area 6 weeks later revealed no evidence of additional cases after the decline in September.

### AGE DISTRIBUTION

The incidence of illness in the various age groups is shown in Figure 135. The highest percentage of cases of aseptic meningitis occurred in those 5 to 9 years old. On the other hand minor illnesses occurred with equally high frequency among the children in both of the younger age categories. In general the age distribution for the two types of illness is similar. Approximately two thirds of the cases occurred in the age group under 20. Less than a quarter of the cases occurred among individuals between 20 and 50 and the cases were extremely rare after the age of 50.

### ETIOLOGIC STUDIES

#### VIRUS ISOLATIONS

Specimens were collected from 152 individuals in Cerro Cordo County. These consisted of 133

TABLE 96. FREQUENCY OF COXSACKIE B 5 VIRUS ISOLATIONS\* BY TYPE OF ILLNESS

GROUP	NUMBER OF INDIVIDUALS	NUMBER POSITIVE	PER CENT POSITIVE
Aseptic meningitis	49	31	63
Minor illness	46	23	50
Well family contacts	44	18	41
Well noncontacts	13	2	15
Total	152	74	49

\* Stool, throat washings, CSF.

TABLE 97. FREQUENCY OF COXSACKIE B 5 VIRUS ISOLATION FROM STOOLS AMONG SICK AND WELL PERSONS BY AGE

AGE	SICK		WELL		TOTALS	
	NUMBER TESTED	PER CENT POSITIVE	NUMBER TESTED	PER CENT POSITIVE	NUMBER TESTED	PER CENT POSITIVE
0-19	67	55	27	64	94	57
20 and over	29	45	23	17	52	33

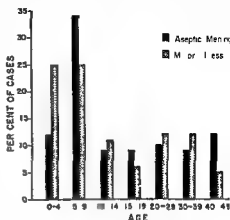


FIG. 135. Per cent distribution of all cases by age.

stools, 46 throat washings and 16 spinal fluids. Sixty-seven strains of Coxsackie B 5 were isolated from 133 stools, 16 from throat washings and 2 from spinal fluids. The frequency of isolations according to type of illness is summarized in Table 96. The virus was recovered from 63 per cent of the patients with aseptic meningitis, from 50 per cent with minor illness and from 41 per cent of the family contacts. Two of 13 individuals who gave neither history of illness nor contact with a clinical case also were found to be excreting the virus in their stools.

The frequency of virus isolation from stools among sick and well individuals in those 20 and over 20 years of age is shown in Table 97. It is seen that there was only a slight difference in the percentage of isolation among individuals in the two age groups. However, among those who had experienced no clinical illness the percentage was considerably higher in the age group under 20 than that over 20 years of age.

TABLE III NEUTRALIZING ANTIBODY RESPONSE TO COXSACKIE B 5 VIRUS\*  
IN PAIRED SERA BY TYPE OF ILLNESS

TYPE OF ILLNESS	NUMBER OF INDIVIDUALS	ANTIBODY					
		ABSENT		PRESENT			
				No Rise†		Rise‡	
		NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
Aseptic meningitis	18	2	11	4	22	12	67
Minor illness	12	2	17	3	25	7	58
Well family contacts	18	5	28	7	39	6	33
Well noncontacts	3	—	—	1	—	2	—
Total	51	9	18	15	29	27	53

\*Against 100 TCD<sub>50</sub>

†Titer of 1:4 or greater with no rise

‡Fourfold rise or greater

## ANTIBODY DETERMINATIONS

Table 98 shows the specific neutralizing antibody response of 51 individuals from whom paired sera were obtained. A fourfold or greater rise in titer to Coxsackie B 5 virus between the first and second serum was demonstrated in 53 per cent of the individuals. Among 18 cases of aseptic meningitis 12 or 67 per cent showed a rise and 4 or 22 per cent had antibodies but without a rise. A rising titer was found in 58 per cent of the patients with minor illness while no change was shown in 25 per cent. No antibodies were found in the remaining 17 per cent. Among the well family contacts 6 of 18 or 33 per cent showed a rise and 39 per cent had antibodies but with no rise.

## DISCUSSION AND SUMMARY

During the summer of 1956 several outbreaks of aseptic meningitis were observed in Iowa. A large outbreak occurring in Iowa was investigated intensively. The results of these studies indicate that the illness was caused by Coxsackie B 5 virus. Illness caused by this virus was also prevalent in the surrounding states of Missouri, Nebraska and Kansas although apparently not in epidemic proportions.

Investigations in Cerro Gordo County indicated that the illness was very widespread involving over 3,000 persons. It appears that the disease was most common in persons under 10

years of age suggesting that older persons may have had an immunity as a result of previous contact with the virus.

The disease was extremely variable in its clinical manifestations ranging from a mild illness to severe illness with central nervous system involvement. Isolation of Coxsackie B 5 virus from a high percentage of the patients with aseptic meningitis as well as from those with minor illness together with the demonstration of specific antibody development suggest that the two illnesses had a common etiology. Detection of the virus and specific antibodies in a high proportion of the family contacts further reflects the prevalence of the virus in the community at the time of the epidemic.

For two consecutive years 1955 and 1956 epidemics of aseptic meningitis caused by two different viral agents occurred in Iowa. These epidemics occurred in two cities, Marshalltown and Mason City located 75 miles from each other. The 1955 epidemic in Marshalltown was due to ECHO virus Type 4 while the 1956 epidemic in Mason City was caused by Coxsackie B 5 virus. These are interesting examples of the periodic appearance of a virus in a given population. The periodic occurrence of viruses is further reflected by the studies conducted in our 6 midwestern states during the past 3 years. In 1954 no Coxsackie B 5 or ECHO Type 4 viruses were isolated from 250 stools from these states. In 1955 ECHO virus Type 4 appeared

in Marshalltown but neither Coxsackie B 5 nor ECHO Type 4 virus was isolated from 300 stools collected from other areas. In 1956 Coxsackie B 5 virus appeared in at least 4 of the midwestern states while only 7 strains of ECHO virus Type 4 were found in 650 stools so far processed. These strains were isolated from two separate family outbreaks in Kansas.

It is evident that these viruses tend to be at times widespread and then disappear entirely from the picture.

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# Encephalomyocarditis Caused by Coxsackie Viruses

DR JAMES H S GEAR

In a graveyard in England on the tombstone of a newborn baby there is the epitaph, "Since I am so soon done for I wonder what I was begun for." Shakespeare did not write that but it is specific nevertheless.

Outbreaks of illness occurring in maternity homes or similar institutions are a well known phenomenon. Among such infections are diarrhoea, pneumonia and myocarditis of the newborn. In several recent outbreaks of myocarditis neonatorum the cause of the infection has been identified as a Group B Coxsackie virus.

The first outbreak occurred in Johannesburg, South Africa, in October and November 1952. Ten newborn babies became ill while in or soon after their discharge from the maternity home in which they were born (Table 99). Six of them died and on postmortem examination the cause of death was found to be an acute focal but extensive myocarditis.

The illness showed a tendency to be diphasic. The first phase was relatively mild and passed unrecognized. In one of the fatal cases the infant refused its food and was found to have a temperature from 102° to 104° F. In some cases these symptoms were associated with upper respiratory catarrh and in other cases with the passage of loose stools containing mucus. After

1 to 3 days the fever subsided. This baby appeared to be well until the onset of the second phase 2 to 5 days later when signs of severe illness, particularly the signs of myocarditis and circulatory collapse, developed.

The second outbreak affected 3 babies in another maternity home also in Johannesburg, in December 1952. In addition to presenting signs and symptoms of myocarditis these babies also showed clear evidence of encephalitis.

The third outbreak occurred in Southern Rhodesia. Three babies were affected. The evidence which was first obtained that the Johannesburg cases were due to Coxsackie B virus was the pathologic lesions observed in newborn baby mice.

Figure 136 is the temperature chart of one of the Southern Rhodesian babies who recovered. It is of interest to note that there is a suggestion of a fever somewhat reminiscent of malignant tertian malaria.

Figure 137 is the temperature chart of the second Rhodesian baby who recovered.

Figure 138 is the temperature chart of the third Rhodesian baby who died. A postmortem examination was carried out on this baby and Figures 139 to 142 show the lesions which were observed. In the heart (Fig 139) there was a

TABLE 99 OUTBREAK OF MYOCARDITIS NEONATORUM AMONG NEWBORN BABIES IN JOHANNESBURG, OCTOBER AND NOVEMBER 1952

CASE NO.	BIRTH	ONSET OF ILLNESS	DEATH
1	October 8	October 15	October 20
2	October 14	October 19	Recovered
3	October 17	October 24	November 4
4	October 19	November 5	November 5
5	October 22	October 27	October 31
6	October 22	November 8	November 9
7	October 29	November 7	November 7
8	October 30	November 18	Recovered
9	October 31	November 8	Recovered
10	November 1	November 7	Recovered

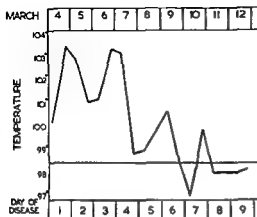


FIG 136 Temperature chart of Baby W born February 25 1954

focal myocarditis shown as a cellular infiltration which was not clearly defined from the surrounding tissues. On high power (Fig 140) it was noted that there was some destruction of the muscle associated with an inflammatory cell infiltration mostly of the histiocytic monocyte type. The lungs (Fig 141) showed marked edema of the pleura but no inflammation; there was marked congestion of the alveolar capillaries but no inflammation, although microscopically it looked as though there was intense inflammation. The liver showed congestion but no clear

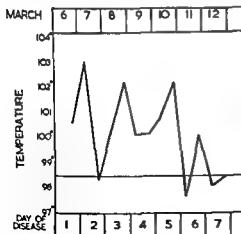


FIG 137 Temperature chart of Baby T born February 26 1954

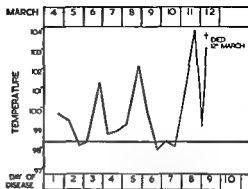


FIG 138 Temperature chart of Baby B born March 2 1954. This case terminated fatally.

evidence of focal lesions. The kidney did not show any characteristic lesions. There was some congestion. The suprarenals (Fig 142) showed intense congestion of the medulla which looked as though it was hemorrhagic, although the pathologists had some doubt as to whether they were hemorrhages or merely intense congestion. In addition, there was an inflammatory cell infiltrate in the medulla.

Mice inoculated with the feces of 2 of the babies and with the fecal contents of the baby who died developed the characteristic lesions of the fat pad. Some mice also showed a focal myocarditis which is characteristic of some strains of myocarditis in baby mice and somewhat reminiscent of the lesions seen in the human babies.

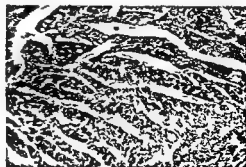


FIG 139 Low power magnification of section of heart muscle showing inflammatory foci (X).

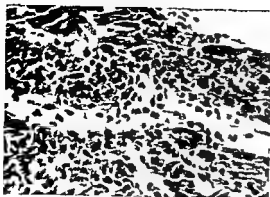


FIG 140 High power magnification of section of heart muscle showing inflammatory degeneration and fragmentation of muscle fibers associated with inflammatory cell infiltrate ( $\times 300$ )

In the Johannesburg outbreaks and in the Southern Rhodesian outbreaks although we obtained evidence of Coxsackie B virus infection we did not prove that the myocarditis was caused by this virus because we did not isolate the virus from the heart muscle. In a more recent case which occurred in Johannesburg in 1955 a 9-day-old baby was admitted to the Transvaal Memorial Hospital for Children and died on the day of admission. On post mortem examination the baby was found to have again the very characteristic lesions of the heart muscle the focal extensive myocarditis. From the heart of this baby a Coxsackie B virus was isolated.

From the outbreak in Johannesburg in 1952 a Coxsackie B3 virus was isolated from the outbreak in Southern Rhodesia a Coxsackie B 4



FIG 141 Section of lung showing marked congestion of alveolae and edema of pleura ( $\times 75$ )

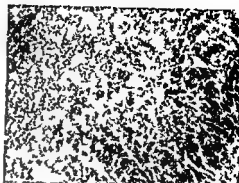


FIG 142 Section of suprarenal showing inflammatory foci in medulla ( $\times 15$ )

virus was isolated from this last case it was a Coxsackie B Type 2 virus which was isolated. So it seems that Coxsackie B Types 2, 3 and 4 have been incriminated in causing this condition. Convincing confirmation of the etiologic role of these viruses has recently come from the workers in Holland. Professor van Crevel and his colleagues and Professor Verlinde and his colleagues who are on the panel and will discuss further the role of these viruses.

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## DISCUSSION

DR CONTRERAS I shall comment on one point that has been clearly presented here namely complexity of antigenic structure within ECHO virus types

It has been shown that in ECHO Type 6 besides the prototype D Amori strain there are strains called 6 prime and 6 double prime that show a broader antigenic constitution than the prototype This fact may have a definite immunologic implication

Will you forgive me for presenting a completely theoretical example? Suppose for a moment that an inactive vaccine for this ECHO Type 6 is needed and we have no information about the occurrences of the subtypes Obviously from the facts presented by the previous speakers the immunologic response elicited by Type 6 would give little or no protection against infection by Type 6 When we give an inactive vaccine we give the necessary antigenic mass once and for all and there is no chance that by further multiplication of the virus in the organism that particles of different antigenic structure could come into play as might eventually occur when an active virus vaccine is given I do not say that an active virus poliomyelitis vaccine should be used because first evidence presented here in favor of the inactive virus vaccine is satisfactory secondly no such complex antigenic structure within polio virus types is known to date Nevertheless I shall describe briefly one strain which we have studied in our laboratory

We have been actively engaged in epidemiologic surveys in Chile plus a few samples coming from Argentina and Brazil On the whole we have tested a few hundred stool samples mainly in HeLa cell cultures adapted by us to horse serum some time ago From these samples we have isolated 288 virus strains of which 218 are polio viruses distributed in the following way 80 per cent Type 1 15 per cent Type 2 5 per cent Type 3 Of the remaining 70 strains good evidence indicates that the great majority are ECHO viruses some are Coxsackie viruses which we have not been able to correlate with any particular disease There are also a small

number that are adenoviruses and one other strain we have studied thoroughly The possibility that this strain namely Virus 285 is a mixture of polio virus Type 1 with another virus cannot be completely excluded so far and we are reinvestigating this point If it does prove that it cannot be separated into two different viral components it may become an interesting Type 1 strain which could have a similar relation to Prototype 1 as the one exhibited by the ECHO 6 to its homologous prototype The importance of such strains in the immunization against poliomyelitis is obvious and would tend to emphasize the fact that a diagnostic unit should work side by side with the laboratory preparing vaccine a unit that should keep track of enteric viruses especially among vaccinated persons

Besides the definite pathogenic role played by enteric viruses their complex antigenic structure lends them a particular interest

DR SVEDMYR Dr Melnick in his lecture pointed out that the polio the ECHO and the Coxsackie viruses share many of their properties and indeed in personal communications he has hinted at the possibility of getting a recombination between polio viruses and ECHO viruses Therefore it may be of interest to mention some observations obtained with a complement fixation reaction on human sera that may indicate the existence of an antigenic relationship between polio viruses and ECHO virus Type 6

Most of the ECHO 6 excretors studied by us were found among cases of aseptic meningitis during an outbreak of that syndrome in Stockholm in 1954—a year with an extremely low incidence of poliomyelitis All excretors of Type 6 virus developed neutralizing antibodies and almost all developed complement fixing antibodies against this virus The majority showed a significant rise in titer with at least one of these reactions thus about 50 per cent in the CF titer There was no significant difference whether the CF antigen was used live or heat inactivated

However the majority of the Type 6 cases also had comparable titers against heated polio virus antigens of all 3 types and about 50 per cent again showed significant rises in titer although the rises tended to be somewhat lower than with a homotypic antigen. Rises were observed with polio antigens even in cases lacking in their convalescent sera neutralizing antibodies against the 3 types of polio virus. Actually CF tests with heat inactivated polio antigens gave a much higher proportion of rises in infections with ECHO 6 virus than in polio infections.

This crossing phenomenon seems to work in the opposite direction too. Thus the majority of the children so far tested who were excreting polio virus Types 1, 2 or 3 had CF titers against ECHO 6 antigen whether the latter was used live or heat inactivated. The titers were comparable with those obtained with heat inactivated polio antigens. The presence or absence of neutralizing ECHO 6 antibodies among these polio cases made no difference. The response was that seen in poliomyelitis cases with the group type heat inactivated polio antigens that is only a few rises in titer were observed.

The virus butchets used as antigens in these tests were specifically neutralized by prototype antisera and the heat inactivated polio antigens as usual reacted type specifically in a CF test with monkey hyperimmune sera. The results seem to suggest that the viruses in question are somewhat related antigenically. The fact that the antibody response as measured by the CF reaction seems to be broader in human beings than in test animals corresponds to similar phenomena within other virus groups like influenza and Coxsackie.

I know that Dr. Johansson has been able to confirm some of these observations and I believe also extend them to some other enteric viruses.

Dr. HAMMOND. In the preceding papers considerable emphasis has been placed on the role of Coxsackie and ECHO viruses in aseptic meningitis and I in myocarditis. Dr. Wenner mentioned that he had made serologic tests on several patients to rule out the presence of Western Equine and St. Louis viruses also. Where certain of the arthropod borne viral encephalitis are present these in their milder

clinical forms present difficult differential diagnostic problems in connection with aseptic meningitis. Also they produce aseptic meningitis. In many years of experience in the field in seeing clinical cases of Western Equine and St. Louis encephalitis and in attempting to establish a laboratory diagnosis we found that many cases could not be diagnosed in the laboratory as Western Equine or St. Louis encephalitis or mumps or poliomyelitis and we presumed that other agents were responsible. We know more about those agents today.

It has been emphasized that the sharp dividing lines are rapidly disappearing between poliomyelitis, Coxsackie and ECHO viruses. Clinically we know that there are difficulties in making differential diagnosis at least in the milder cases called aseptic meningitis. Several speakers have suggested that paralytic cases might be caused by these other viruses. I would certainly not be surprised if in the many strains that will be worked with in the future with the many individual persons with different susceptibilities that severe cases with paralysis would be found due to these other viruses.

It has been pointed out that aseptic meningitis apparently has been caused by ECHO viruses Types 4, 5, 6, 9 and 14. Additional work by Kilbourne and Enders has also indicated that ECHO virus Type 16 can produce an epidemic of aseptic meningitis. Coxsackie viruses 1, 9, B, B3, B4 and B5 have also been pointed out as being responsible for these diseases. Two speakers have mentioned some difficulties with ECHO Type 13 insofar as an antigenic relationship with ECHO Type 1 is concerned. This virus was isolated in our laboratory in the Philippines from Air Force personnel and when we distributed it to other persons apparently this was a mixture of ECHO Type 1 and ECHO Type 13. Of this we are quite convinced from recent work that we have done. Apparently we have been able to separate this mixture now. Whether on original isolation this was a mixture or whether this was contamination in repeated passage in the laboratory we are not yet sure. We have reisolated the virus slowly and we are not certain. However both Type 1 and Type 13. We are certain that plaques have been picked from the mixture for both Type 1 and Type 13. We are certain that there is a Type 13 that is antigenically distinct



Dr MACCALLUM The behavior of infectious diseases in island populations is often of interest and we thought that the information available on ECHO 9 virus infection in Great Britain would be a suitable contribution to this discussion. The viruses isolated were identified by cross neutralization tests with the prototype strain and antiserum. It is interesting to note that after passage in tissue culture one of the strains isolated in 1955 and 1956 produced myositis and paralysis in newborn mice.

In August and September 1954 9 babies were admitted to a hospital in east London each suffering from a similar type of illness which also developed in a baby already in the hospital with a chest infection. The most frequent signs of illness were irritability, fever, vomiting, diarrhea and a discrete reddish brown maculopapular rash on the face, the trunk and the extensor surfaces of the limbs and the soles of the feet which lasted from 3 to 14 days. Reddening of the fauces and enlargement of the superficial lymph nodes were also present. Although protein, sugar and chlorides in the cerebrospinal fluid were all within normal limits there was an increase in the number of cells varying from 10 to 376 per cu. mm. of which 75 to 100 per cent were lymphocytes. The illness was not serious in any of the babies. Stools were received from 6 of the babies, acute and convalescent sera from 7 and single convalescent sera from 2. No specimens were received from the first case seen. Macrae at the Virus Reference Laboratory Colindale isolated a strain of virus from 5 of the 6 stools by inoculation of tissue cultures of monkey testes and monkey kidney. The 5 strains of virus were found to be similar to each other and none were virulent for suckling or adult mice, monkeys, guinea pigs or rabbit even after passage in tissue culture. They were not identified by Macrae as being related to ECHO 9 virus until 1956. Rising titers of neutralizing antibodies to the new viruses were found in sera of 6 babies and high titers in convalescent sera from 7 others. No virus was isolated from the stool nor antibodies found in sera from 1 baby. There was no clinical evidence that older siblings of the investigated babies had been affected but no laboratory tests were carried out on them. General practitioners in the area did not remember having seen any groups of similar cases. Two lots of

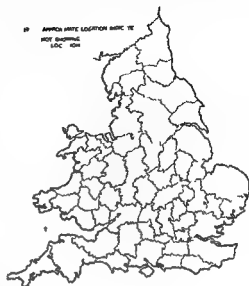


FIG. 143. ECHO 9 virus infection in Great Britain.

gamma globulin prepared from pools of plasma collected in 1953 from adult donors from London and surrounding areas contained low titers of neutralizing antibody for the virus.

Although this laboratory continued to carry out large numbers of isolation tests in monkey kidney cultures on stools from patients with aseptic meningitis there was no indication of the presence of ECHO 9 virus or similar cases of aseptic meningitis with rash until August 1955. It is true of course that an insufficient number of specimens was tested from all parts of the country to say that infection with ECHO virus Type 9 was not occurring. From August until December 1955 cases of aseptic meningitis, some with a rash, were recognized in East Anglia with outbreaks in the Cambridge area. ECHO 9 viruses were isolated from the stools, the cerebrospinal fluid and the throat washings and there was a rising titer of neutralizing antibody to ECHO 9 viruses in paired sera from patients in the Cambridge, Ipswich and Leiston areas.

In 1956 investigations of cases of nonparalytic poliomyelitis and aseptic meningitis continued and the first case of ECHO 9 virus infection detected was in a patient from north London in April. As far as we know the first definite outbreak commenced in Nottingham in

June In July single cases were detected from County Durham in the north to Surrey in the south as well as many places in between with outbreaks in Lancashire and Leicestershire and possibly other places. The disease continued to be prevalent in these areas and new cases appeared in many others so that by the end of November numerous cases of aseptic meningitis some of which had a rash had been seen in a high proportion of counties of England and Wales and virus was isolated from about 20 of them (Fig 143). I understand that ECHO 9 viruses were also isolated from similar cases in Scotland.

At the time of coming to Geneva in 1957 in July there had been no reports of the presence of ECHO 9 virus illness.

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- PROF DR NIHOUL A characteristic epidemic of aseptic meningitis which occurred in Belgium in 1956 was proved to have been caused by ECHO virus Type 9. Many of the Belgian ECHO 9 strains are not pathogenic for suckling mice on inoculation of material representative of human clinical specimens but acquire such a pathogenicity after one or a few passages in monkey kidney tissue cultures. Based on other similar observations as Dr Melnick suggested one might consider for purposes of classification the host range of the virus as it naturally exists in human material rather than the host range obtained with strains manipulated in the laboratory. However such a criterion in relation to ECHO 9 strains does not seem quite reliable since we have now observed that the few human fecal specimens and one spinal fluid which contained ECHO virus Type 9 were indeed able to provoke histologically detectable myositis in suckling mice on direct inoculation.
- Similar to the observations of Dr Wenner and Dr Melnick we found antigenic differences between the original ECHO 9 strain from the United States and the European strains of ECHO 9. This fact and the pathogenicity for suckling mice indicate that this group of viruses are heterogeneous but we did not find any antigenic variation between the different European strains of ECHO 9 which so far have been examined by ourselves. So several Belgian strains isolated at the beginning and the end of the outbreak from different places in Belgium and strains from the Netherlands and Switzerland all were similar when compared in a neutralization test against specific rabbit immune serum.
- During the study of the Belgian outbreak of aseptic meningitis we observed 14 cases of atypical evolution of the disease. Indeed in those cases there were clinical signs of involvement of the brain and/or of the spinal cord with paralysis. In 9 of these cases mixed infection with both polio virus and ECHO 9 virus was demonstrated. In 4 cases by isolation of both viruses and in 5 cases by isolation of one virus and demonstration of a rise of antibodies.
- In the 5 remaining cases no virus was isolated but the clinical picture was proved but the

presence of polio virus could neither be proved nor disproved. However analysis of the results suggests that the simultaneous presence of ECHO virus Type 9 and polio virus in the same individual might be more than merely due to chance.

The detection of a double infection may be come facilitated by the availability in the future of tissue cultures which would be specifically susceptible to infection with ECHO viruses but not to polio viruses. We have observed that trypsinized mouse cells are not susceptible to ECHO viruses Type 1 through Type 14. Neither cytopathogenic effect nor multiplication was observed in such tissue as kidney from mice or in kidney muscle or brain from mouse embryo. These results are in marked contrast with what was observed *in vivo*. In suckling mice good multiplication of the Belgian ECHO 9 strain was detected in the muscles.

Cells in continuous cell culture are not susceptible to ECHO viruses. Attempts are now in process to maintain cells in tissue culture originating from kidneys of cynomolgus embryo. This culture has been maintained for only 45 days and has undergone 5 subcultures. Up to now these cultures have not yet passed through the critical phase. In their present status these cells are still as susceptible to ECHO viruses as was the primary culture.

PROF DR VERLINDE. The 1956 outbreak in the Netherlands of aseptic meningitis due to ECHO 9 virus was extensive and covered practically all regions of the country. Family infections were common. Virologic and serologic examination revealed that not only a syndrome of meningitis was due to ECHO 9 virus but also that minor illness and a rubelliform rash were common, the latter especially in children.

This outbreak of meningitis was particularly puzzling for clinicians because of a simultaneous outbreak of poliomyelitis. In individual cases it frequently was impossible to differentiate on a purely clinical basis between nonparalytic poliomyelitis and minor illness or meningitis due to infection with ECHO 9 virus.

In 1956 the poliomyelitis unit in my laboratory recovered over 1 500 virus strains of these more than 600 were strains of the ECHO group mostly ECHO 9. About 100 were of the Coxsackie group. Our group is now collecting the

clinical data of the patients examined which is a very difficult and time-consuming job. The complete clinical data of 400 patients with a clinical diagnosis of meningeal involvement with or without rash are now available and more will become available before long. From these only 10 per cent yielded polio virus and 46 per cent ECHO 9 virus. The ECHO strains have been recovered mostly from stools but also from rectal swabs, throat swabs and cerebrospinal fluid. These materials have been routinely examined by inoculation of trypsinized monkey kidney cell cultures and suckling mice.

All strains that took directly in suckling mice proved to be Coxsackie Group A or B viruses. All strains that were isolated in monkey kidney culture proved to be poliomyelitis Coxsackie Group B or ECHO viruses. The ECHO strains failed to produce any cytopathogenic effect in HeLa cells.

The ECHO strains which were neutralized by ECHO Type 9 serum could be divided into two groups according to their pathogenicity for experimental animals. Although none of them had been isolated by direct inoculation of suckling mice, approximately 70 per cent produced lesions resembling those of Coxsackie A in these animals following inoculation of tissue-culture passage virus.

Circumstantial evidence has been obtained that the ECHO Type 9 viruses are responsible for the outbreak of meningitis and sometimes even meningo-encephalomyelitis with transitory paralytic attacks. First because of the fact that examination of acute phase and convalescent serum samples from patients as well as from family contacts revealed the development of neutralizing antibodies as evidenced by significant rises in titer. Second because of the fact that a Type 9 ECHO virus has been isolated from the intestinal contents as well as from the aseptically removed medulla of a child who died from encephalitis. Quantitative virologic examination of the medulla revealed a virus titer of  $10^{4.5}$  per gram of tissue.

So far 2 strains of ECHO-9 virus, one pathogenic for suckling mice, the other nonpathogenic for suckling mice, have been examined for their pathogenicity for cynomolgus monkeys. Both strains failed to produce clinical signs of illness following intracerebral and intraspinal inoculation of normal monkeys although discrete

lesion were found in the central nervous system. In cortisone-treated monkeys, however, the suckling mouse pathogenic strain produced paralysis.

Histologic examination of 4 infected monkeys after cortisone treatment showed an intense lymphocytic meningitis covering practically all levels of the central nervous system. Moreover, in several levels of the spinal cord perivascular infiltrations consisting mainly of mononuclear cells, a more or less diffuse or local glial proliferation and a small accumulation of leukocytes were found predominantly in the anterior horns but also around the central canal and even in the posterior horns. Although a few nerve cells showed degenerative changes and neuronophagia, most of these cells appeared to be normal, unlike in poliomyelitis. Similar lesions to a lesser extent were found in several parts of the brain, for instance the pons, the cerebellum, the thalamic region and other parts of the brain stem.

In cortisone-treated monkeys inoculated with a suckling mouse nonpathogenic ECHO 9 virus, only a few discrete lesions were found in the pons and other regions of the brain stem.

Hence it appears that the viruses of the ECHO group, at least those associated with diseases simulating poliomyelitis, deserve further attention.

However, there seems to be little doubt at least during the mixed epidemic of poliomyelitis and viral meningitis associated with ECHO 9 virus in the Netherlands that mixed infections with both viruses in the same individual were not uncommon. First, from several patients both viruses have been isolated at different occasions; second, the complement fixation reaction with poliomyelitis antigen might indicate that a considerable number of mixed infections has occurred.

Although the complement fixation reaction has been performed in a limited number of the 400 patients from whom complete clinical data are available, it is suggested that in approximately half of the patients from whom only ECHO virus has been isolated, the poliomyelitis complement fixation reaction with heated Type 1 antigen was positive. This may suggest either a mixed infection or an antigenic relationship between ECHO 9 virus and Type 1

polio virus. Nevertheless, it seems that ECHO Type 9 virus is an important etiologic agent in aseptic meningitis and in other forms of illness simulating certain forms of poliomyelitis.

**DR SIGURDSSON.** Outbreaks of meningitis occurred in Iceland in the period from July to December 1956. The reporting was by no means complete. In Reykjavik, for instance, it is known that the incidence was several times higher than the reports showed. In spite of that, in one of the medical districts the incidence was almost 10 per cent.

The age incidence was distributed fairly evenly over the various age groups. The clinical signs and symptoms were those common for meningitis. No deaths occurred. One hospital in Reykjavik admitted 29 of the more severe cases. Fever lasted for from 2 to 10 days; in two thirds of the cases it was from 38° to 39° C. and in one third of the cases from 39° to 40° C.

No bacteria were found in the spinal fluid of these patients. No virus could be recovered from spinal fluid or feces in HeLa cell cultures or in baby or adult mice. Twenty-two samples of spinal fluid were inoculated into monkey kidney tissue cultures. A virus strain for these cells was isolated from 7 of these samples. Twenty-nine samples of feces were tested in the same way and 7 strains of a similar virus were isolated. The 14 isolated strains seemed to be similar as far as can be seen.

Five of the new strains were found to be neutralized by convalescent sera from the patients from whom they were isolated but not from acute sera from the same patients. The convalescent serum samples from each of these patients also neutralized the virus strains isolated from the other patients. This is an indication that the virus strains are immunologically related or identical. Two other strains isolated from spinal fluid paralyzed and killed baby mice. On the other hand, the strains tested were not neutralized by antisera to polio virus Types 1, 2 or 3. Attempts to neutralize these strains with antisera to ECHO Types 6 and 9 have not given conclusive results. Type 6 antiserum did not neutralize but there was some neutralization by Type 9. These strains have been provisionally grouped as ECHO strains.

This evidence suggests that widespread epidemics of meningitis caused by ECHO virus

TABLE 100 DEMONSTRATION OF VIRUSES IN SPECIMENS FROM PATIENTS WITH PARALYTIC POLIOMYELITIS, ASEPTIC MENINGITIS AND OTHER DIAGNOSES AT THE HOSPITAL OF INFECTIOUS DISEASES OF ESSENSTINA IN THE SUMMER AND THE FALL OF 1954

AGE	TOTAL NUMBER EXAM	COXSACKIE VIRUSES			PER CENT	POLIO VIRUSES	PER CENT	ECHO VIRUS TYPE 6	PER CENT TYPABLE VIRUSES	PER CENT	TOTAL NUMBER POSITIVE SPECIMENS
		A	B	A							
Paralytic poliomyelitis	0-15					2	100				
	>15					1	100				
	Total					3	100				
Meningitis	0-15	2	5	5	13	1	3	73	58	2	33
	>15		3		8			21	57		23
	Total	2	8	3	10	1	1	44	57	2	56
Others	0-15							5	38		5
	>15							1	6		1
	Total							6	20		6

0 - specimens with both infectious mononucleosis and human embryonic lung tissue



TABLE 101 COXSACKIE VIRUS ISOLATIONS FROM PATIENTS ADMITTED TO DIFFERENT HOSPITALS IN SWEDEN FROM APRIL TO NOVEMBER 1954 DIAGNOSED AS ASEPTIC MENINGITIS AND OTHERS  
A FEW COXSACKIE NEGATIVE CASES OF PARALYTIC POLIOMYELITIS ARE EXCLUDED

AGE	DIAGNOSIS	NUMBER EXAM	A 4	A 5	A 7	UNTYPED PROBABLY A STRAINS	PER CENT A STRAINS	B 4	PER CENT B STRAINS
<15	Aseptic meningitis	181		1	16	10	15	11	6
	<5 Cells in C S F	59			1	5	10	3	5
	Others								
	L P not performed	23		2		2	17	1	4
>15	Aseptic meningitis	129			4	3	5	9	7
	<5 Cells in C S F	111			1		1	6	5
	Others								
	L P not performed	66	1	1			3	3	5
	Total	569	1	4	22	20	9	33	6

occurred in Iceland in December of 1956. The reported incidence in affected districts was 370 cases per 100 000. In one district it was as high as 10 per cent of the population. The reported figures are known to be too low. Our observations therefore confirm the findings reported from other countries.

Dr JOHANSSON. In 1954 in a study of an outbreak of aseptic meningitis together with Dr Lycke we isolated ECHO 6 in a high percentage. I want to stress the importance of using different ways of isolations. For example by using a combination of human embryonic lung trypsinized monkey kidney and suckling mice it was possible to find a probable causal agent in 73 per cent (Table 100 p 237) indicating that no other virus need be considered. Besides Coxsackie B-4 we also found Coxsackie A 7. The interest in the latter type has increased since Dr Voroshilova isolated the same type from some cases of paralytic poliomyelitis. This type seems to have been rather common in Sweden in 1954 as can be seen in Table 101 where we can see that A 7 was isolated in 9 per cent of children with aseptic meningitis. The material examined does not permit a statistical

evaluation but it strongly supports a correlation between A 7 and aseptic meningitis especially as there was an increasing titer in 6 of the cases. One of the children had rather severe encephalitis symptoms with transient muscular weakness.

As in Western Europe ECHO 9 was extremely common in Sweden in the fall of 1956. Thus I personally observed 3 different outbreaks and furthermore Dr Wesslen has isolated 80 ECHO 9 strains from 300 cases of aseptic meningitis in northern Sweden. The first case from the first outbreak observed was a boy coming from Holland the biggest epidemic was along the Baltic Sea in northern Sweden where the shipping communications with Holland Belgium and England are very pronounced. The epidemic is still going on and it has now spread all over the country.

One of the outbreaks I studied in more detail. In this outbreak using both embryonic lung and monkey kidney tissue ECHO 9 was isolated in 83 per cent. Of 14 fecal samples tested in both kinds of tissue 86 per cent was positive in lung tissue and 36 per cent in monkey kidney. Among 20 lung positive samples Dr Wesslen

TABLE 102. FREQUENCY OF ISOLATIONS OF ECHO VIRUS TYPE 4 BY TYPE OF ILLNESS

TYPE OF ILLNESS	NUMBER EXAMINED	NUMBER POSITIVE IN FLIES	COMMENTS
Aseptic meningitis + exanthema	8	4	One case also positive in C.S.F.
Aseptic meningitis	4	4	One case also positive in C.S.F.
Minor illness	1	1	Lambda puncture not performed
Healthy family contacts	3	3	
Total	16	12	

found all but one to be negative in monkey kidney. This figure seems to indicate that our strains were more sensitive for human embryonic lung than for monkey kidney tissue.

Primary isolation of ECHO 9 is negative in suckling mice. In my material about two thirds were positive after isolation in tissue culture by injection by the subcutaneous route in mice. Only one was positive by the intracerebral route. However after one blind passage in mice it was possible to adapt some further strains for mice either by passing directly in mice or through tissue culture. The above mentioned 19 monkey kidney negative strains isolated in human lung by Dr. Wesslen were positive in the former tissue when they were passed from human lung.

The European ECHO 9 strains often have been isolated in cases of aseptic meningitis with a rubellalike rash. Therefore it was interesting to find the same autumn another outbreak of aseptic meningitis with a similar rash from which ECHO 4 could be isolated. As can be seen in Table 102 ECHO 4 was isolated in 75

per cent of the cases and virus was isolated from spinal fluid in 7 cases. Family occurrences with a varying clinical picture were common. The number of positive isolations was the same in lung as in monkey kidney tissue. However it was difficult to demonstrate neutralizing antibodies with the standard technique. There were significant titers (Table 103) in the complement fixation test using a concentrated antigen. Also cross reaction was observed with other ECHO strains and with Coxsackie strains.

Prof. Dr. van Groenou in his paper Dr. Clear cited the clinical, pathological and virologic studies made by my colleagues and me—to which I add the name of Dehling—in 4 cases of myocarditis of the newborn. In all cases a Group B Type 4 Coxsackie virus was isolated from the heart muscle. The third case was important; it concerned 1 of 2 premature infants who were nursed in the same box shortly after each other. Both developed aseptic meningitis with a severe pleiocytosis. A few days later one of the infants died soon after having shown

TABLE 103. OCCURRENCE OF COMPLEMENT FIXING ANTIBODIES AGAINST ECHO VIRUS TYPE 4 BY TYPE OF ILLNESS

TYPE OF ILLNESS	NUMBER EXAMINED	ANTIBODY		
		PAIRED SERA		
		NO RISE	NUMBER	Rise† NUMBER
Aseptic meningitis + exanthema	5	1		4
Aseptic meningitis	3	1		2
Healthy family contacts	2	1		1
Total	10	3		7

† Rise of 1 or greater.  
† Fourfold rise or greater.

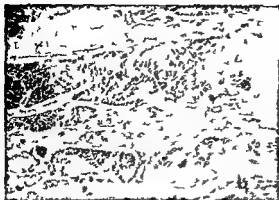


FIG 144 Extensive scarring of heart muscle (medium magnification)



FIG 145 Very distinct lymphocytic cellular infiltrates (high magnification)

symptoms of myocarditis. The microscopic examination confirmed the diagnosis of myocarditis and meningo-encephalitis; foci of inflammation were also seen in the liver. Dr Gear also stressed that the mothers of our patients before or after delivery showed an influenzalike disease with high fever.

In the period when these cases of myocarditis in the newborn caused by Coxsackie virus were observed in the pediatric clinic of the University of Amsterdam, 5 cases with an analogous clinical course were observed in newborns in an obstetric clinic in Amsterdam. No virus studies were made in these cases. Three of the babies died; in 2 an autopsy was made and in both cases a myocarditis and a pericarditis were found. The pathologist, Professor Deelman, investigated the preparations of one case and made the diagnosis of Fiedler's myocarditis, a form of myocarditis which more and more is regarded as being of viral origin. A sixth newborn fell acutely ill outside the obstetric clinic at home on the eighth day of life and died 1 day later. At autopsy of this baby also a Fiedler's myocarditis was found.

Epidemiologically all these cases belonged to the same group as our cases. During the time of this small epidemic of myocarditis in the newborn caused by Coxsackie virus, some cases of myocarditis with lethal course in the newborn were also observed outside Amsterdam.

Recently Dr Ostrowski from Haifa informed me in an epidemic of myocarditis in infants in which more than 50 infants were infected and many of them died occurred in

Haifa and in some cases a Coxsackie virus was isolated from the heart muscle. Dr Falk from the Rambam Government Hospital told me that from January until June of 1971 there were 33 cases and in one case of an acute pericarditis which subsided within a few weeks a Coxsackie B3 virus was isolated from the pericardial fluid by Dr Bernkopf. Therefore the problem is as was also indicated by Dr Gear not at all a local problem. In this connection I must also mention the large number of cases of myocarditis in newborns in Munich described by Stoeber in 1952 although no virus was isolated in these cases.

I agree with Dr Gear about the necessity of a number of measures to be taken when an epidemic of Coxsackie B virus infection in a population occurs—this especially with regard to the possibility of an outbreak of an epidemic of encephalomyocarditis in the newborns with such a large mortality. I stress also the necessity of studying further the possibility of protection of the babies exposed to infection.

Dr Gear also briefly cited the reduction of the resistance of adult mice against infection by Coxsackie viruses by the administration of cortisone. In this connection the work of H. Bourne and co-workers deserves special attention. These authors found that extensive and disseminated myocardial necrosis could be produced in adult mice by simultaneous administration of certain strains of Coxsackie virus and cortisone. The state of intrinsic resistance of the adult mouse is changed in this way to one of marked susceptibility resulting in death. This observation may indeed be of importance for the explanation of

the susceptibility of the myocardium of newborn infants for the Coxsackie virus

The speculation of Kilbourne in this connection on the possible conversion of the benign pleurodynia of adults to a more serious if not fatal disease by the stimulus of endogenous or exogenous corticosteroids surely must be kept in mind and of course it is of very great importance that the adult patient is treated in the clinic for a long time with cortisone

The conclusion seems to be justified that acute myocarditis in older children and adults may be accompanied by far fewer symptoms than in very young infants. The prognosis in babies during the acute stage is severe. Early treatment with digitalis may promote recovery.

When the patient recovers from acute myocarditis restitutio ad integrum takes place but with the exception of diphtheric myocarditis only a few investigations are known. However about the occurrence of the contrary namely enlargement of the heart and decompensatio cordis as late consequences of myocarditis several studies have been published.

My co-worker Dr Hartog and I observed 3 patients with enlargement of the heart and decompensatio cordis in which the postmortem study demonstrated the presence of chronic myocarditis (Figs 144 and 145). These patients died from progressive cardiac failure.

In explaining such cases of chronic myocarditis the possibility of recurrent myocarditis caused by different viruses must be considered. It is difficult provisionally to give a proof of this possibility.

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## Studies on Cultured Mammalian Cells

WEDNESDAY MORNING, JULY 10 1957

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# Nutritional Requirements for Growth of Cells and Viruses

DR HARRY EAGLE

A number of technical developments in the art of tissue culture have made possible exploration of the nutritional requirements and metabolic activities of a wide variety of animal and human cells. Perhaps the most important single advance has been the provision of cell cultures which can be grown either as monolayers adherent to a glass surface and overlaid with a fluid medium or as suspensions of discrete cells. The cells thus are taken out of the tissue and out of a supporting plasma clot and grown in immediate contact with a fluid medium. Their growth rate is increased thereby and their equilibration with the medium facilitated. That medium can be changed at will and the effect of such changes on the rate of cell growth can be determined with precision.

In this manner determination of the nutritional requirements of a variety of animal and human cells deriving from both normal and malignant tissues has been possible. Since large amounts of cells become available for chemical study one can explore the metabolic pathways involved in the utilization of these essential metabolites for the biosynthesis of the cellular macromolecules. The data derived from these studies on cellular nutrition and metabolism in turn provide leads to the mechanism of virus synthesis and to the effects of viral infection on cellular metabolism. Thus we come full circle. The techniques of the microbiologist have been applied to mammalian cells in pure culture and these studies in turn provide new avenues of exploration for the pathways of viral biosynthesis.

## THE AMINO ACID REQUIREMENTS OF CELL CULTURES

If from a medium which contains only the essential requirements for growth one removes a single essential amino acid the cells develop cytopathogenic changes within a few days and ultimately disintegrate and die. In their early stages these degenerative changes are reversible if the missing amino acid is added. Lack to

the medium the cells recover and go on to multiply at a normal rate.

Eight amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, tyrosine, valine) are known to suffice for nitrogen balance in feeding experiments in man. However in cell culture at least 13 amino acids have proved to be essential for the survival and the growth of every animal and human cell so far examined.<sup>1</sup> All have been found to require cystine,\* tyrosine, arginine, histidine, and glutamine\* over and above the 8 essential amino acids.

Only the L-amino acids are active and dipeptides have been found to be as active as their component amino acids indicative of active peptidase activity.<sup>6</sup> Only minor differences have been found between the cell lines derived from various organs or between cells deriving from normal and malignant tissues with respect to the amounts of the various amino acids required for optimal growth,<sup>11</sup> and it is of interest that these maximally effective concentrations *in vitro* closely parallel the serum concentrations of the various amino acids *in vivo*.

The anomalous requirement for 13 amino acids by serially propagated cell cultures when the human organism requires only 8 is not due to the fact that the cells have altered on prolonged cultivation. Monkey kidney cells in first tissue-culture passage tested within 48 hours after their removal from the host before there had been extensive proliferation required the same 13 amino acids as did the lines which have been serially propagated for years.<sup>9</sup>

In view of the uniform amino acid requirements of the cell strains so far studied such minor differences as have appeared deserve emphasis.

1. A rat carcinoma cell has been found by McCoy, Maxwell and Neuman<sup>17</sup> to require asparagin in addition to glutamine.

2. A rabbit fibroblast has been found to require serine.<sup>1</sup>

\*Qualification with respect to the cystine and glutamine requirements as discussed on page 245.

3 A variant HeLa cell proved capable of hydroxylating phenylalanine to tyrosine<sup>14</sup>

4 The human cell cultures so far studied all require glutamine<sup>15</sup> and glutamic acid does not substitute except at extremely high and non-physiologic levels. Recent observations by Dr Robert DeMars in our laboratory indicate that at those high concentrations there is apparently an adaptive formation of glutamine transferase and synthetase. Such adapted cells can then grow for a time in a glutamine free medium containing low concentrations of glutamic acid.

5 Unlike any other cell yet tested monkey kidney cells in first tissue-culture passage can use glutamic acid in lieu of glutamine presumably because of the presence of an active glutamine synthetase<sup>6</sup>

6 In confirmation of the findings of Melnick and his co-workers<sup>7</sup> glycine is highly growth stimulatory for monkey kidney epithelium in first tissue-culture passage<sup>8</sup>

7 Recent experiments in this laboratory indicate that cystine should be removed from the list of the essential amino acids. It is not formed from methionine in tissue culture but as in the case of *Escherichia coli* and as in the case of a water mold studied by Volkhonsky<sup>9</sup> it can be synthesized by every cell strain so far examined from reduced inorganic sulfur compounds and glucose. The pathways involved in that synthesis are under present study.

#### AMINO ACID METABOLISM IN TISSUE CULTURE

The primary function of most of the essential amino acids is for incorporation into cellular protein. With only a few exceptions at physiologic levels there is no significant metabolic breakdown or interconversion<sup>16</sup>. Glutamine constitutes a special case which deserves emphasis because of its special position in virus synthesis. Like any other essential amino acid it is incorporated directly into protein<sup>16</sup>. However in addition it is used for the synthesis of glutamic acid, aspartic acid, proline and asparagin and in a limiting medium both the carbon and the amide nitrogen are used in the biosynthesis of the nucleic acids<sup>17</sup>.

There are 2 additional aspects of amino acid metabolism of significance in relation to virus synthesis. One is the presence in all human cell strains examined of a large cellular pool of

amino acids at concentrations approximately 10 times that of the surrounding medium<sup>18</sup>. The second is the fact that in contrast to bacteria there is active intracellular turnover of protein i.e. the cell protein is constantly breaking down and being resynthesized at a rate of approximately 1 per cent per hour<sup>14</sup>. This intracellular turnover could be of importance in relation to virus synthesis.

#### VITAMIN REQUIREMENTS OF CELL CULTURES

To date only 8 vitamins have been shown to be essential for cells in culture. The first 7 were vitamins of the vitamin B complex (choline, folic acid, nicotinamide, pantothenate, pyridoxal, thiamine, riboflavin). In addition meso-(myo-) inositol known since 1938 to be an essential growth factor for certain yeasts and fungi and long suspected as playing an important role in animal metabolism has proved to be rigorously essential for the survival and the growth of 70 out of 71 cell lines examined<sup>1</sup>. Of the 9 isomers of inositol only 1 myo inositol is active in promoting growth.

Specific vitamin deficiencies can be produced in cell cultures by withdrawing a single vitamin and these deficiencies can be cured by the restoration of the missing factor. The conjugated cofactors (vitrocrum factor, diphospho- and triphospho-pyridine nucleotide, coenzyme A, pyridoxal, coarboxylase and flavin mono- and dinucleotide) substitute for the corresponding vitamins<sup>1</sup> their occasionally low activity relative to the parent vitamin presumably reflects a difference in cellular permeability.

#### CARBOHYDRATE REQUIREMENTS IN CELL CULTURES

With respect to carbohydrates glucose is essential for cell survival and growth in culture both as a source of energy and as a metabolite for the synthesis of the nutritionally nonessential amino acids (glycine, serine and alanine), carbohydrates, lipids and nucleic acids. However it is not uniquely active. Every cell so far studied has proved to be capable of utilizing fructose and mannose in lieu of glucose similarly many can use galactose and ribose and a number of bacteria have also proved to be active. While differences have been observed with respect to the pattern of metabolism with these



various sugar substrates. With glucose and mannose there is an active aerobic glycolysis the amount depending on the concentration in the medium. However with other substrates and in particular with galactose there is but little fermentative metabolism and the pH of the medium remains relatively stable during the growth of the culture. Correspondingly relatively little of these substrates is metabolized in the process of growth.<sup>8</sup>

Thus a total of 28 nutritional factors have been shown to be required for the survival and the growth of most mammalian cells in culture: 13 amino acids, 8 vitamins, glucose and 6 ions ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{HPO}_4^{--}$ ).<sup>9</sup> In addition a small amount of serum protein must be added to the medium. The nature of the essential component(s) supplied by the protein remains to be determined.

### VIRUS PROPAGATION

What bearing do these results have on propagation of viruses? Of the 28 components essential for the survival and the growth of animal and human cells, how many are similarly essential for viral propagation? To what extent can the cell draw on its own components for viral synthesis rather than on the medium? Are the viral protein and nucleic acids synthesized *de novo* from the amino acids and nucleotides of the cellular pool or are the cellular proteins and nucleic acids partially degraded and reutilized? Only a few of these questions can be answered now although one may reasonably hope that additional information will develop rapidly.

The nutritional requirements for viral synthesis have been worked out in detail in one model system, the poliomyelitis virus growing in the HeLa cell. In this model system the only components of the medium required for viral synthesis other than salts were glucose and glutamine.<sup>10</sup> In a medium containing all the essential amino acids other than glutamine, all the essential vitamins, salts and serum protein supplemented with the 6 nonessential amino acids, free ammonia, purines and pyrimidines

but lacking glucose and glutamine the amount of virus elaborated by HeLa cells was reduced to one ten thousandth of the amount formed in a complete medium. Conversely in a medium containing only glucose, glutamine and salts, and lacking all of the other components required for cell survival, essentially normal amounts of virus were formed.

In this model system the cell clearly forms virus at the expense of its own cell substance. The virus proteins are formed in large part either from a pre-existing amino acid pool or by the breakdown of cell protein; similarly the virus nucleic acids are formed either from the nucleotide pool or by the breakdown of the cell nucleic acids. There is no present information as to whether the role of glucose and glutamine in the propagation of poliomyelitis virus by the HeLa cell consists in the maintenance of the metabolic processes of the cell or whether they are used directly for virus synthesis.

Although glutamic acid substitutes for glutamine in the elaboration of virus, the concentrations necessary for maximum virus output were the same as those necessary for the growth of the cell; this despite the fact that the experiments are carried out in a nongrowth medium comprising only glucose, glutamine and salts.<sup>1</sup> Furthermore with monkey kidney cultures which can make glutamine from glutamic acid and significant amounts of glutamic acid from glucose or from other amino acids, neither glutamine nor glutamic acid was required for the maximum elaboration of poliomyelitis virus.<sup>1</sup>

Also with glucose and fructose the concentrations necessary for the maximum propagation of virus were the same as those necessary for the optimum growth of the cell, even in a nongrowth medium.<sup>1</sup> The virus yield with other sugars is under continuing study.

Thus similarity in the concentrations required for cell growth and for virus propagation so far is limited to glucose and glutamine. It does not extend to any of the other amino acids nor does it extend to the vitamins. Dr. James Darnell and I have recently observed that the depletion of the cellular reserves of nicotinamide, thiamine and riboflavin to the point of cessation of growth and extensive degenerative change has had no important effect on the capacity of the HeLa cell to form poliomyelitis virus. However pyridoxal deficiency resulted in

<sup>10</sup> Bicarbonate is not necessary as the rate of  $\text{CO}_2$  exchange is sufficient to maintain the pH. Needle is to say this does not exclude its nutritional role. Metabolism in the absence of added bicarbonate comes available to the cell by the oxidation of glucose. The role of trace elements in the nutrition of a cell remains to be elucidated.

a marked reduction in virus output and the virus forming capacity of the cell could then be restored by the addition of a full complement of amino acids

The quantitative and perhaps qualitative differences in the requirements for cell growth and for virus propagation are illustrated further in the effects of various inhibitors. Cyanide and dinitrophenol at concentrations which caused the early death of the HeLa cell had no immediate effect on its capacity to form polio virus

Clearly animal cell cultures provide an elegant and flexible tool not only for the study of cell metabolism but also for the study of the complex process of viral propagation. In comparison with the large body of information with respect to bacteriophage what has been learned to date is only the beginning of the beginning but we may look forward confidently to a period of rapid progress in this fascinating and important area

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# Clones from Single Mammalian Cells

DR THEODORE T PUCK

Mammalian biology during the last half century has dealt to a large extent with structure and functions of tissues and organs. While it has been recognized for approximately three quarters of a century that the ultimate unit of these structures is the cell, most of the methods for study of mammalian phenomena have dealt with many-celled populations. Detailed morphologic, physiologic and biochemical information has accumulated elucidating specific functions of many tissues and cell aggregates. Yet in almost no case is it clear to what extent any given cell is autonomous and to what extent its behavior is governed by its status as a subunit of a particular organ itself tightly integrated into the overall economy of the body. Thus the degree to which individual cells of specialized tissues have retained the capacity for unlimited growth, the extent to which different cell specializations represent changes of genome or of nongenetic apparatus, the potentialities for further specialization or for reversal of differentiation possessed by a particular cell type, the nature of stable self-reproducing and even symbiotic association of cells and viruses which may be possible, the possibilities for genetic modification by processes like random mutation, directed transformation, transduction and sexual exchange of nuclear or cytoplasmic genetic determinants, and the extent to which processes like cancer formation and ageing are manifestations of genetic or physiologic processes localized within individual cells, have remained largely unknown.

The last 4 decades have witnessed tremendous advances in understanding of genetic and physiologic processes in a wide variety of cells of independent micro-organisms. Much of this progress was due to the availability of a simple technique whereby every single cell plated in a semisolid nutrient medium multiplies in isolation to form a macroscopic colony. In our laboratory a program was initiated to develop a similar methodology for mammalian cells in

hope that the concepts and the operations of quantitative microbiology and microbiological genetics then could be applied to the individual cells of the mammalian body.

A plating technique for mammalian cells completely analogous to that used for *Escherichia coli* or *Neurospora* was first achieved with the aid of a feeder layer of  $\times$  irradiated cells. Our experiments were guided by the fact that early developments in tissue culture had resulted in routine methods for growth of large inocula of mammalian cells. In more quantitative studies, Earle and his co-workers demonstrated specifically the need for mutual co-operation between cells in a massive inoculum. These investigators showed that isolated cells did not multiply in a large volume of the nutrient media then employed. However, single cells when mixed in a capillary tube which conserves diffusible products produced self-sustaining colonies. Reasoning from these considerations, we devised a method whereby single HeLa cells were added to a petri dish containing nutrient medium plus 100,000 additional cells which had been  $\times$  irradiated so as permanently to block their own multiplication. These irradiated cells formed a feeder layer for the viable cell inoculum. This technique permitted every one of the cells of the viable inoculum to multiply and form a macroscopic colony as shown in Figure 146. The plating efficiency achieved by this means closely approximates 100 per cent and the reliability and the precision of the results compare favorably with the most quantitative techniques available for micro-organisms like *Escherichia coli* or T2 bacteriophage.

Studies then were initiated to elucidate what function is performed by the irradiated "feeder" cell layer in the hope that it could be fulfilled in other ways and the need for the feeder cells thus eliminated. It was found that the feeder cells can function in 3 ways: (a) they can compensate for specific molecular and nutritional deficiencies in the medium; (b) they can neu-



FIG 146 Demonstration of how a feeder cell system operates in promoting growth of colonies from single human cells (Top) Plate with irradiated feeder cells alone (actual size) (Bottom) Identical plate as above but to which were added 100 unirradiated cells. The colony count obtained was 9.

tralize toxic agents like antibodies that may be present in the medium which always contains a mammalian serum component and (c) the feeder layer may permit clonal growth of cells which otherwise fail to multiply because of trauma suffered in the course of dispersal into the single state. By methods involving excessive mechanical or chemical trauma. When all of these functions are fulfilled by specific adjustments of the nutrient medium and by a sufficiently gentle dispersal procedure the need for feeder cells is eliminated and excellent growth with 100 per cent plating efficiency is obtained.



FIG 147 Growth of colonies from single cells under conditions where the need for feeders has been eliminated. These are cells from a single clonal strain (S-3) of the HeLa cell originally from a human carcinoma of the cervix.

From single cells plated directly into petri dishes containing only the appropriate nutritional medium. A typical example is shown in Figure 147. In our experience so far, newly established cell strains frequently require feeder systems at first for high plating efficiencies of the single cells to be achieved. After cultivation in the laboratory, which always includes progressive adjustment of the composition of the nutritional medium, good plating efficiencies almost always achieved without the need for feeder cells. Elimination of the need for feeder cells probably represents a combination of cell adaptation to growth in vitro as well as better matching of the cell's nutritional needs by the adjusted medium. It is important to note that with the new nutrient solutions which we have developed it is often possible to obtain clonal growth of single cells isolated directly from tissues of human subjects.

#### APPLICATION OF SINGLE-CELL PLATING TO VARIOUS HUMAN SOMATIC CELLS

These procedures have been applied successfully to human cells originating from a large variety of organs. Excellent plating efficiencies have been achieved with cells representing any of the following conditions of origin: young or adult and various normal or cancerous organs.

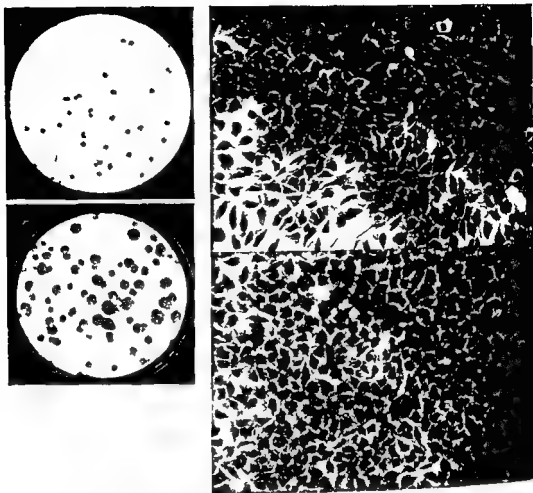


FIG 149 Growth of colonies with epithelial morphology from single cells obtained from a variety of noncancerous human organs (Top left) Liver cells (normal size) (Top right) Photomicrograph of a single liver cell colony ( $\times 170$ ) (Bottom left) Conjunctival cells (normal size) (Bottom right) Photomicrograph of a single conjunctival cell colony ( $\times 200$ )

newly isolated cells or strains which have been long established in tissue culture morphology resembling that of either epithelial or fibroblast forms and either normal diploid or polyploid chromosomal condition. The plating procedures here described have proved to be successful with cells from every human organ which has been tested including liver spleen skin kidney conjunctiva cervix appendix amnion and bone marrow. Figures 148 and 149 present the appearance of colonies arising from the plating of single cells of various human organs by means

of these techniques. Clonal cell lines may be established readily by picking any well isolated colony exactly as is done with single colonies of bacteria or single plaques or bacteriophage.

These techniques permit 3 new types of operations with mammalian cells. It makes possible identification of the particular cells in a given population which are capable of sustained multiplication under the defined conditions. Since each single cell can multiply in isolation this method also permits ready isolation of rare mutations whose presence would be obscured in op-



FIG. 149 Typical colonies with fibroblastlike morphology from normal human organs (*Left*) Colonies from human spleen cells (actual size) (*Right*) Enlargement of colony arising from single cell of human spleen ( $\times 75$ ) Cells are spindle shaped and elongated

erations involving large populations and whose divergent behavior then can be used as markers to illuminate genetic processes in mammalian somatic cells. Finally it provides a more quantitative and reliable means than was previously available for measuring cell multiplication and the influence thereon of various agents.

#### ANALYTIC STUDIES OF CELL GROWTH

The use of this technique to permit more quantitative study of cellular reproduction is illustrated by the growth curve of Figure 150 which is obtained readily by daily counts of the number of cells in about 70 developing colonies of a plate originally seeded with single cells. The initial lag period during which the cells presumably adapt to the medium as well as the subsequent linear logarithmic increase in numbers are complete counterparts of the growth curve of *E. coli*. The construction of such a curve for cells growing under different conditions of nutritional stress or action of physical or chemical agents permits localization of different kinds of actions on the growth cycle. Thus some agents reduce the fraction of cells in the population which can multiply others merely increase the lag period. Still others may change

the slope of the logarithmic part of the curve and finally others may cause the curve to become horizontal after a given amount of growth has been accomplished. Various combinations of these actions also occur. Studies of the dynamics of mammalian cell growth and the effect thereon of substances with differential action on different cells should profit materially from the use of this technique.

In this connection every human cell we have studied so far appears to achieve the same limiting multiplication rate as its growth conditions are steadily improved. This maximal rate for sustained reproduction corresponds to a

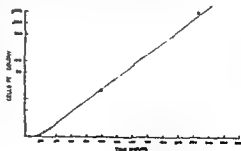


FIG. 150 Typical growth curve of single HeLa cell in complete nutrient medium

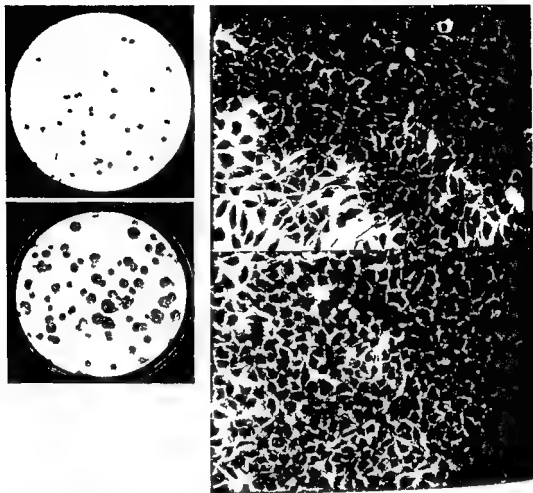


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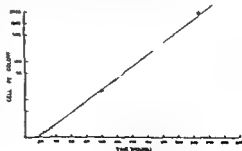


FIG. 150 Typical growth curve of single HeLa cell in complete nutrient medium



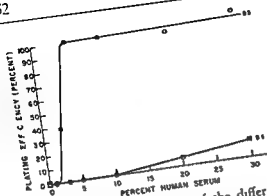


FIG 151 Demonstration of the difference in growth response to human serum of two different mutants which were isolated from the population of the HeLa cell strain obtained from a human cervical carcinoma. Use of a basal synthetic medium plus 5 per cent serum gives 100 per cent plating efficiency of the single cells of S-3 and zero for S-1 cells. Yet massive inocula of either cell will proliferate even in the low serum medium.

generation time of 18 hours. All but about 40 minutes of this period represents the intermitotic phase.

### STUDIES ON CELL NUTRITION

Many different investigations attempting to define the nutritional needs of mammalian cells have been described and on the basis of these studies certain key molecular requirements for sustained growth have come to be recognized as common to a variety of mammalian cells cultured *in vitro*. However, usually these investigations utilized growth of massive cell inocula. The growth requirements obtained by such methods often are uniform for cells obtained from widely different tissues and even for cells which have different morphologies. Thus Eagle in his most illuminating studies found as you have just heard that a specific nutritional medium is adequate for support of many kinds of cells. Such results appear to be disappointing in the hope that mammalian cells cultivated *in vitro* might display specific biochemical properties reflecting different functions in the body.

When the single-cell plating procedure is employed to study nutritional requirements of a variety of human cells, new nutritional requirements often emerge. With this technique a variety of differentiated patterns of nutritional require-

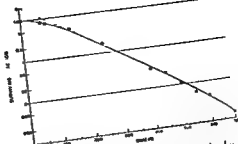


FIG 152 X-ray survival curve of a human somatic cell. This particular curve is obtained with the HeLa cell as well as with all of the noncancerous human cells so far tested.

ments has been found. Reproducible differences in molecular growth requirements have been demonstrated between morphologically identical cells arising from the same human tissue specimens and from morphologically similar and different cells obtained from different tissues of the same individual. Thus, for example, the same individual cell lines with clearly demonstrable nutritional differences have been found naturally occurring in the population constituting the HeLa cell strain, one of the most widely used lines of tissue culture. The data of Figure 151 demonstrate that a basal synthetic medium, supplemented with 5 per cent human serum, can produce 100 per cent plating efficiency of one strain and no growth whatever of the other.

The reason behind the differences in molecular requirements for growth exhibited by single cells on the one hand and massive inocula on the other is of enormous biologic interest. Earle first suggested that the decreased nutritional requirements of massive inocula is a reflection of an inability of the biosynthetic apparatus of mammalian cells to keep pace with the loss of diffusible metabolites. It would follow from these considerations that differences in nutritional requirements for growth exhibited by single cells from different tissues but not by large cell aggregates is a reflection either of differences in membrane permeability or in the rates of biosynthesis attainable by the various cell types. Studies of the differences in metabolic pattern of single cells as compared with cell populations of varying compositions promises to afford an approach to systematic investigation of different kinds of cell-cell interaction.



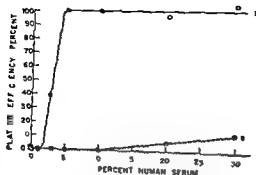


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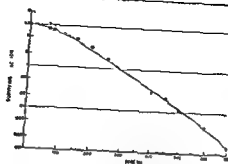


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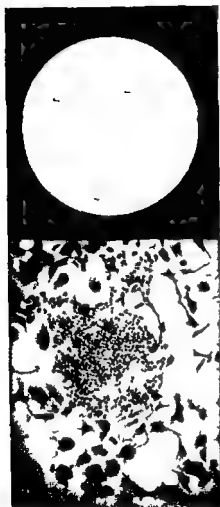


FIG 153 Demonstration of giant production from human cells by x irradiation. (Top) Plate seeded with single cells and then irradiated with 900 r. Only two actual colonies developed. The remaining cells became giants each of which is visible to the eye (actual size). (Bottom) Photomicrograph of a normal colony growing among the radiation inactivated cells which are forming giants ( $\times 75$ )

in the mammalian body tissues. Under specified conditions single cells may exhibit molecular biochemical requirements for growth which have not been found heretofore in bacteria. An example of such action is the need exhibited by single

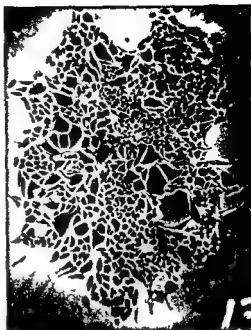


FIG 154 Typical colony from the bizarre mutant of S-3 produced as a result of x irradiation. Each single cell when plated in nutrient medium produces a colony studded with monster forms as shown

cells of the HeLa S3 clone for cholesterol. The quantitativeness of the single-cell plating technique permits titration of cholesterol by a bio-assay with human cells in a manner completely analogous to that used with the B vitamins with bacteria.

#### GENETICS AND THE ACTION OF IONIZING RADIATION

This method has been used to measure the action of ionizing radiations on mammalian cells and has permitted determination of their x ray survival curve as shown in Figure 152. These experiments have yielded values of the mean lethal dose of radiation for various human cells varying between 96 to 160 r, a quantity much lower than that which had been presumed to represent the toxic radiation level of x rays for somatic cells. Curves obtained for cells from 4 different organs including both normal and neoplastic types are very similar. An abundance

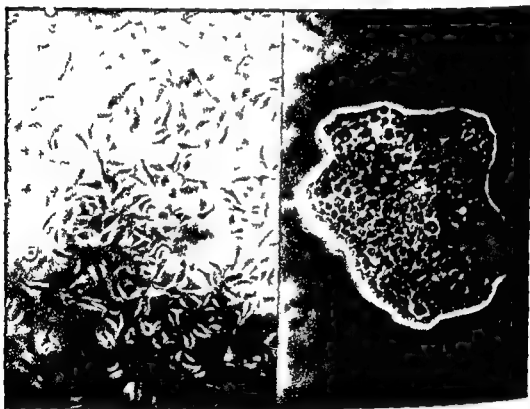


FIG 15: Demonstration of the effect of human serum in producing highly stretched migratory spindle shaped cells instead of compact polygonal epithelial like cells in colonies of S-3. The pair of photographs on the left (top and bottom) represent plating of S-3 cells in a medium containing 20 per cent human serum. The pair of photographs on the right (top and bottom) show the results of plating exactly the same number and kind of cells in a medium containing no human serum but 10 per cent of horse or porcine serum. All other conditions were identical in the two platings. The photomicrographs represent the appearance of typical colonies taken from each plate.

of evidence indicates that the process primarily responsible for the destruction of the cell's reproductive power is a damage in the genetic apparatus.

Calculation shows that cell death occurs on the average after absorption of a total quantity of x rays equivalent in energy to a temperature rise of only one ten thousandth of a degree centigrade. Hence it is not surprising that these cells whose reproductive powers have been destroyed by x irradiation continue to metabolize abundantly carrying out extensive biosynthesis. Such cells continue to grow without reproduction forming giant cells readily visible to the unaided eye (Figs 153 and 154). These giants constitute a fascinating biologic population with many interesting properties including an enormously increased susceptibility to virus attack. While these giant cells have never been observed to reproduce they can carry out many specific functions including phagocytosis of vital dyes and specific biosynthesis of many viruses. From unirradiated populations and from survivors of irradiated cell inocula new clonal stocks have been isolated with behavior sufficiently different from the wild type to permit their designation as mutants. Some of these new stocks display characteristically different growth requirements from the wild type (Fig 155). Others are characterized by different colonial morphologies also shown in Figure 155. Studies aimed at isolating mutant markers useful in genetic investigations are in progress.

#### APPLICATION TO STUDIES OF CELL DIFFERENTIATION

By means of these techniques study has been carried out on clonal cell stocks isolated from a variety of normal tissues obtained from a single individual. It has proved to be possible to isolate stable lines from the skin which consistently grow only in the fibroblastlike form even after many generations of *in vitro* cultivation. Single lines plated in any medium available if they grow at all invariably produce typical fibroblastlike colonies. Other clonal cell lines obtained from the lung are completely epithelial in morphology and equally stable and consistent in the expression of this characteristic. Finally clonal cell lines have also been isolated from the lung which were typically fibroblastlike in morphology throughout clonal isolation and for

approximately 20 generations of growth thereafter but which then changed their form to an epithelial like morphology.

In addition we have found that certain epithelial cells can change their form to simulate a fibroblastic morphology in a completely reversible fashion as a result of addition in the medium of certain fractions of human serum. These experiments suggest that the single-cell technique may be helpful in unraveling the wide variety of variation mechanisms available to mammalian cells which undoubtedly play important roles in differentiation.

#### VIRUSLIKE INTERACTION

The use of single-cell plating has been adapted to measurement of the lethal effect of viruses on cell reproduction. The ability of a virus particle to destroy a cell's colony forming capacity has been made the basis of a new method for titration of virus particles and for measurement of the process of virus penetration into its host cell. Various aliquots of a virus can be added to a series of tubes containing identical cell suspensions. After virus attachment the single cells are plated and the colony forming efficiency is scored. This method provides a new quantitative assay of a separate virus function.

One of the most interesting of our observations made possible by the single-cell plating technique was that mammalian cells can become carriers of an otherwise lethal virus without loss of their ability to multiply indefinitely as apparently healthy cells. This state is achieved in a small proportion of the cells when a large population is infected with virus. The cells in which this virus-carrier state is achieved continue to reproduce normally and form laboratory stocks which have been stable for more than 2 years or 100 generations. When plated as single cells 60 to 70 per cent of this population forms normal microscopic colonies. However when these same cells are plated on top of a layer of x irradiated giant cells which are particularly susceptible to destruction by virus action each carrier cell produces a plaque in which the giants are completely disintegrated. A further parallelism between these cells and the lyso-genic virus-carrying bacteria lies in their immunity to the destructive action of an inoculum of free virus sufficient to annihilate the parent cell.

## SUMMARY

The single-cell plating technic makes possible application to mammalian cells of quantitative operations that have proved fruitful in the physiologic and genetic virus studies of microorganisms. These tools may provide new approaches to many of the classical biologic problems.

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# Alterations in Clonal Populations of Monkey Kidney Cells

DR RAYMOND C PARKER

In recent years numerous reports have concerned the development of strains of altered cells that can be propagated *in vitro* indefinitely. Some of the alterations occurred gradually, others suddenly. Occasionally they resulted in round cells with a tendency to live and multiply in suspension even in stationary cultures. At other times they yielded morphologically uniform populations of cells with a tendency to live and multiply while attached to the glass. In most instances the altered cells cannot readily be identified with any cell type existing in the body. Fortunately however they usually can be centrifuged washed and propagated by quantitative culture procedures. The altered cell strain that is best known is Earle's L strain derived from the subcutaneous connective tissue of a normal C3H mouse. Before alteration the cells were cultivated for long periods in plasma clots treated regularly with horse serum and chick embryo extract. Eventually some of the substrains were exposed to methylcholanthrene. Over a period of 1 to 3 months after the addition of carcinogen to the cultures cells of some of the substrains lost their fibroblastlike appearance became rounded and granular and many of the altered strains including strain L, consistently produced tumors when injected into mice and certain of the control strains also became altered and produced tumors on injection.

In previous communications from our laboratory we described cell alterations that have occurred in cultures of normal and malignant cells from many sources including normal kidney epithelium from mouse rat rabbit monkey and man. It is the purpose of the present report to review the facts already known about cell alterations in cultures of monkey kidney epithelium and to describe efforts now being made to understand certain chromosomal changes observed in cultures of monkey kidney epithelium both before and after cell alterations have occurred.

In 1953 we prepared a series of cultures from chopped monkey kidney and carried them in fluid medium consisting of horse serum chick embryo extract and balanced saline. After several weeks great showers of round cells (Fig 156 top left) appeared in many of the cultures and when isolated they had a tendency to multiply in suspension even in stationary cultures. When they were propagated in tubes rotated at 50 to 60 rpm according to the method of Graham and Simionescu it was possible to achieve a hundredfold increase in the logarithmic phase of multiplication without an initial lag period. During the past 3 years many additional experiments have been made from freshly explanted monkey kidney cultivated in synthetic media supplemented with horse serum. In many of these cultures altered cells sometimes appeared as early as 51 days and as late as 107 days after explantation. With 2 exceptions in which free living round cells were obtained the altered cells (Fig 156 top right and bottom left) remained adherent to the glass of stationary cultures and resembled Earle's L cells. In 1 experiment in which some of the cells were subcultured at 14 22 37 and 53 days respectively altered cells appeared between the 51st and 60th day after explantation regardless of the number of times they had been subcultured. Figure 156 top right shows a nest of altered cells with the unaltered kidney epithelium around the edge. Figure 156 bottom left shows altered cells at the upper right and several unaltered epithelial cells at the lower left. In another experiment monkey kidney epithelium was kept alive for 6 months in a chemically defined medium (No 858) without protein supplement of any sort but alterations were not obtained. Figure 156 bottom right shows the healthy appearance of the epithelial cells at 91 days prior to which the cells had been subcultured twice. Because all of the alterations that have been described up to this point occurred in cultures containing mixed cell populations the possibility was not excluded that they were due to the select



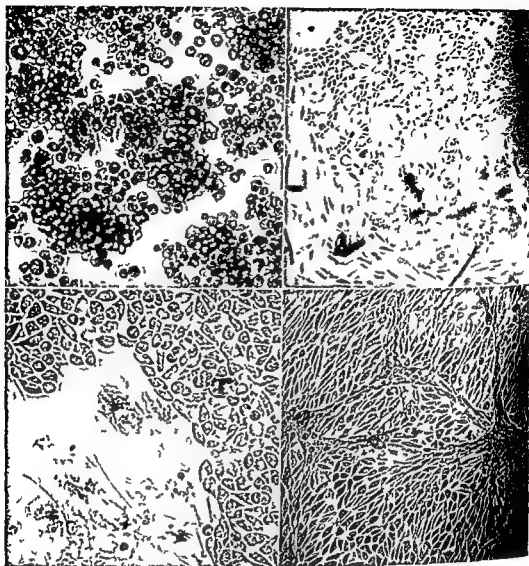


FIG 156 (Top left) Altered cells derived from mixed cell culture of monkey kidney tissue after 82 days in 40 per cent horse serum and 3 per cent chick embryo extract in balanced saline. Even in stationary cultures these cells tend to multiply while unattached to the glass (Culture 19017) ( $\times 180$ ) (Top right) Nest of altered cells in 67-day-old culture (20739 10) prepared from freshly explanted monkey kidney tissue and maintained in CMRL-857<sup>18</sup> supplemented with 20 per cent horse serum. Note unaltered epithelium surrounding nest of altered cells which contains several giant cells ( $\times 38$ ) (Bottom left) Altered cells (upper right) and several unaltered epithelial cells (lower left) in culture (C181 1) prepared from trypsinized suspension of monkey kidney tissue after 120 days in CMRL-857 supplemented with 20 per cent horse serum during which time it was subcultured once ( $\times 200$ ) (Bottom right) Unaltered epithelial cells in a culture (C.77) of a series that was prepared from freshly explanted monkey kidney tissue and had been maintained for 91 days (at the time this photograph was made) in unsupplemented chemically defined medium CMRL-858<sup>19</sup>. During this period the series had been subcultured twice ( $\times 100$ )

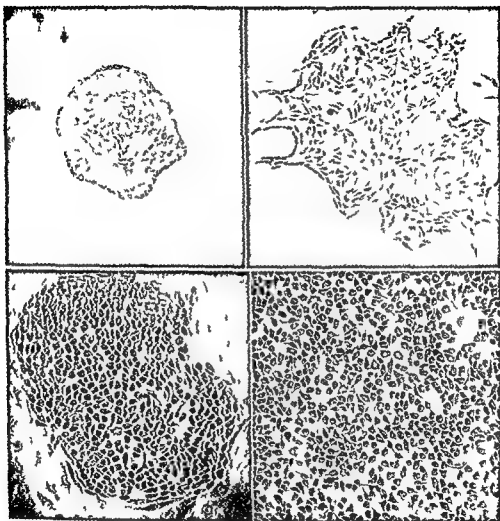


FIG. 157 (Top left) Clone of monkey kidney epithelial cells 8 days after a single cell had been isolated in a capillary drop of medium on a coverslip under a layer of oil in a Petri dish. The medium which was renewed twice during this period was CMRL 1046 supplemented with 20 per cent horse serum (Culture 21798-9) ( $\times 38$ ) (Top right) Another clone of monkey kidney cells 11 days after a single cell had been isolated under the conditions described for Culture 21799 (Culture 21800-7) ( $\times 38$ ) (Bottom left) Clone of monkey kidney epithelial cells undergoing cellular alteration. The clone was left on the coverslip for 13 days then transferred to a 13  $\times$  100 mm test tube 16 days before this photograph was made. Note unaltered epithelial cells surrounding nest of altered cells (Culture 21877) ( $\times 100$ ) (Bottom right) Cells from one of several nests of altered cells that developed in the clone shown at the bottom left. This subclone (Culture 21936-3) had been maintained for 6 days in a Carrel flask when this photograph was made ( $\times 100$ ).

tion of particular cell types present in the original explants. To eliminate this possibility it was necessary to observe the phenomenon in clonal populations derived by single-cell isolation. Accordingly single cells from cultures of monkey kidney epithelium were isolated in capillary drops of medium on a coverglass under a layer of oil in a 60 mm petri dish according to a technique devised by deFonbrune and adapted to animal cell work by Lwoff and collaborators. Examples shown are one such clone 8 days after the single cell had been isolated on the coverglass and another clone that was photographed on the tenth day (Fig 157 top left and right). Both consist of typical epithelium. The medium which consisted of CMRL-1066 supplemented with 20 per cent horse serum was always passed through fritted glass filters after the addition of the serum. The developing clones were left in the capillary drops with 2 changes of medium a week until they were large enough to be transferred to flasks or tubes. By these means then 330 clones have been developed that survived for a week or more. Of these 330 clones 27 per cent sur-

vived for at least 1 month 12 per cent for at least 2 months and 6 per cent for at least 3 months. Altered cell strains (Fig 157 bottom left and right) were derived from 21 of these clones and the average time required for the alterations to occur was 33 days after the single cell isolation or 41 days after explantation from the monkey. The most rapid cell alteration occurred 19 days after the single-cell isolation or 29 days after explantation from the monkey. The slowest alteration occurred 81 days after explantation. These experiments proved conclusively that the appearance of the new cell type was not due to the selection of a particular cell explanted from the organism. Appropriate care was always taken of course to eliminate the possibility of contaminating the clones with cells from other sources.

From time to time over the past few years, various strains of altered monkey kidney cells derived from mixed cell populations have been tested for polio virus susceptibility but always with negative results. It could not have been known in any of these experiments whether or not the immediate progenitors of the altered

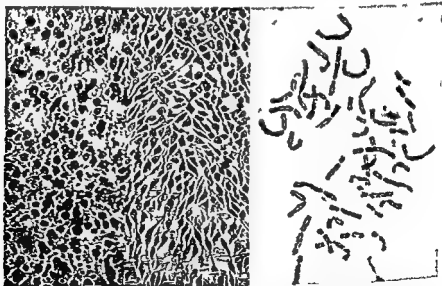


FIG 158 (Left) Unaltered monkey kidney epithelial cells in culture shown in Figure 157 bottom left photographed 4 days after infection with polio virus. The cells are completely destroyed ( $\times 112$ ). (Center) Altered cells in culture shown in Figure 157 bottom left photographed 4 days after infection with polio virus. The cells are not visibly affected by the virus ( $\times 117$ ). (Right) Photomicrograph of a typical metaphase plate (diploid) of an unaltered cell in a culture of monkey kidney epithelium ( $\times 1125$ ).

cells would have been susceptible but once alterations were obtained in developing clones it became possible to study the direct effect of the alteration process on virus susceptibility. In an experiment of this sort a newly established clone developed as epithelium. On the 13th day when a fair sized sheet of cells had formed on the coverglass they were detached with trypsin and transferred to a small stationary test tube where the survivors continued to multiply slowly to form epithelial sheets as before. Then 16 days later several nests of exceedingly active altered cells (Fig 157 bottom left) appeared in the epithelium and cells from one of these nests (such as you see here) were removed with a capillary pipette and transferred to a larger container where they continued to multiply with great rapidity (Fig 157 bottom right) as you see here but the transforming culture itself which contained both the altered and the unaltered cells was then treated with the MEF<sub>2</sub> strain of polio virus at a concentration of  $2 \times 10^4$  plaque forming particles per ml. After 7 days the virus had severely damaged the unaltered cells. By 4 days when the unaltered cells were completely destroyed (Fig 158 left) the altered cells (Fig 158 center) still appeared to be healthy and active. This experiment proved conclusively that the change in susceptibility of the population to polio virus occurred during alteration.

It might be helpful at this point to summarize the information that has been obtained by observing cell alterations many times in cultures of monkey kidney epithelium. Thus it is known that

- 1 The appearance of altered cells in a culture population is not due to the selective advantage of a particular cell type present in the original explants
- 2 Altered cells from monkey kidney differ morphologically from the parent-cell type but are remarkably similar morphologically to altered cells from other sources
- 3 Altered cells may appear in a healthy young population or in an old one in which multiplication of the parent-cell type has ceased. They continue to multiply indefinitely even in cultures in which the parent-cell type disappears. They are capable of unlimited survival.

4 Altered cells may appear in relatively small populations. One clone became altered on the 26th day when there were only 30 surviving cells.

5 Altered cells from monkey kidney have never been observed to revert to the parent-cell type. Of course this stability could be due to the selective advantage of the altered cells.

6 Altered cells may be propagated continuously under conditions that are not favorable to unaltered cells. Thus altered cells from monkey kidney epithelium unlike the parent-cell type may be propagated in agitated suspensions.

7 Altered cells from monkey kidney epithelium unlike freshly isolated monkey kidney epithelium are either not susceptible or only slightly so to polio virus. Also it has been found by Drs E S Lennor and J S Kaplan of the University of Illinois that altered cells from monkey kidney cultures are less sensitive to diphtheria toxin than are the unaltered cells.

Altered cells from monkey kidney epithelium differ from unaltered monkey kidney cells both in chromosome number and in chromosome morphology. Unaltered monkey kidney cells have 47 chromosomes all of which are metacentric and most of which can be identified individually. Figure 158 right shows a normal altered monkey kidney cells were examined by the colchicine-orcein technic which will be described in more detail later. The number of chromosomes in each of 100 nuclei of one such monkey clone are shown in Figure 159 where

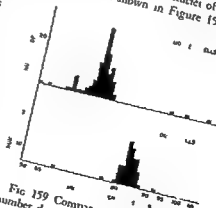


Fig 159 Comparison of chromosome number distribution in altered cells derived from monkey and marmoset kidney epithelium

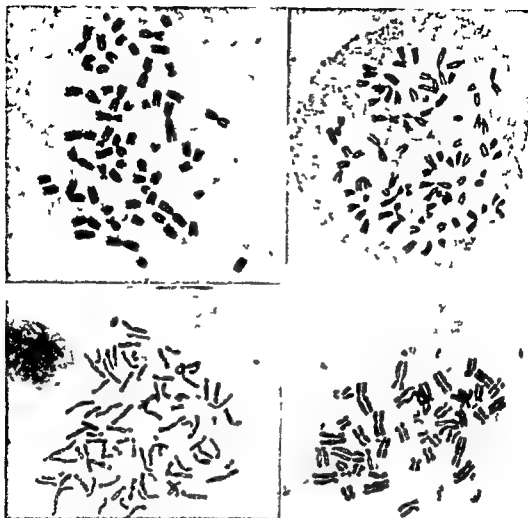


FIG 160 (Top left) Photomicrograph of a typical metaphase plate of an altered cell from a clone (22) derived from monkey kidney epithelium ( $\times 1000$ ) (Top right) Photomicrograph of a typical metaphase plate of an altered cell from a culture strain derived from mouse kidney epithelium ( $\times 1000$ ) (Bottom left) Photomicrograph of a typical metaphase plate (tetraploid) of an unaltered cell in a culture of monkey kidney epithelium ( $\times 1000$ ) (Bottom right) Photomicrograph of a 4 stranded tetraploid metaphase plate of an unaltered cell in a culture of monkey kidney epithelium ( $\times 1000$ )

It will be seen that the distribution is essentially unimodal with a peak at 69 chromosomes. Other clones that were examined showed a similar distribution of chromosome numbers. A typical altered chromosome complement is shown in Figure 160 top left where 18 of the chromosomes are metacentric, the remainder acrocentric. In clear preparations an extremely short arm can always be seen in chromosomes of this type; the centromeres are not strictly

terminal. Table 104 shows a more refined analysis of a subclone in that it gives the frequency distribution of the number of metacentrics in nuclei of a given chromosome number. Thus, for example, of 17 nuclei with a total 69 chromosomes, 4 had 17 metacentrics, 9 had 18 metacentrics, 3 had 20, and 1 had 21. This information illustrates something of the heterogeneity that one obtains even in cultures arising from a single cell. When other clones and subclones were ana-

# Alterations in Clonal Populations of Monkey Kidney Cells

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TABLE 104 DISTRIBUTION OF METACENTRICS  
IN NUCLEI OF DIFFERENT CHROMOSOME  
NUMBER (MONKEY KIDNEY CLONE 22)

TOTAL NUMBER OF CHROMOSOMES	NUMBER OF METACENTRICS					
	15	16	17	18	19	20 21
59						
60		1				
61						
62				1		
63			3			
64			1			
65	1			1	1	
66			3	4	1	
67			4	3	1	
68			5	2		
69	2		1	1		
70			4	7	4	
71	1		10	1	1	
			9		3	
			5	7	7	
			1		1	

lyzed the frequency distribution of the number of metacentrics was similar to those found in this clone. Also when we examine the distribution of total chromosome numbers in a typical subclone we find that the mode and the range of chromosome numbers is similar to those of the parent clone. When the cytologic analysis was refined still further certain marker chromosomes could be distinguished among the metacentrics and the acrocentrics but even with respect to these marker chromosomes all clones and subclones that were examined were remarkably similar.

The results of these studies have indicated that all carefully examined clones and subclones are identical with respect to modal chromosome number, approximate range of chromosome numbers, morphology of chromosomes and the presence of distinctive marker chromosomes. This essential identity of chromosome pattern rules out the possibility that altered cells arise from random breaks and recombinations of chromosome fragments even if one grants the unlikely assumption that this particular alteration confers a strong selective advantage. We are left then with only two possibilities either we are dealing with a directed induced chromosome change of a kind and specificity so far unknown or cellular contaminations at some level have been responsible for the similarity of these clones. Though we have been aware of the danger of cellular contaminations throughout

all of our work we cannot at this moment exclude it as a possibility. These studies were made in collaboration with Drs L. Siminovich, Department of Microbiology, School of Hygiene and K. H. Roehls, Department of Botany.

Because of simultaneous studies made by other colleagues in Toronto namely Drs J. A. Axelrad and E. A. McCulloch of the Ontario Cancer Institute it is possible to compare the chromosome changes associated with alterations in monkey kidney cultures with those that occur in cultures from the mouse. Newly established cultures of kidney epithelium from C57 mice were found to have a chromosome complement similar to that described for normal diploid mouse cells. The vast majority of cells had 40 chromosomes all of which were acrocentric. But 3 independent strains of altered mouse cells were found to be similar to each other and distinct from altered monkey kidney cells in that the modal chromosome number was 83 of which 15 to 17 were metacentric. A typical altered cell complement is shown in Figure 160 top right. Figure 159 is a histogram of one of the altered mouse strains (below) shown in comparison with that of an altered monkey strain (above). Within the last few weeks my colleagues in Toronto have found still another chromosome picture associated with alteration in a culture of kidney cells from a 70-day-old chick embryo. In this case the modal chromosome number was 67 to 64 with 11 to 14 metacentrics. Up to the present then we have encountered 3 distinct categories of altered cells with respect to their chromosome complement: (1) the monkey kidney type (?), the mouse kidney type and (3) the chick kidney type. It should also be mentioned that Levan has reported changes in chromosome number and morphology in 2 of the human strains of cells developed by Chang.

The alterations that have been described occurred only after prolonged cultivation of the cells. It could not have been known whether the chromosome changes occurred at the time of isolation or that transitional changes may have occurred earlier. Accordingly and again in collaboration with Dr. Siminovich and Dr. Potl, an effort was made to follow the chromosomal constitution of monkey kidney cells as soon as possible after explantation from the animal. While no striking changes were seen that could

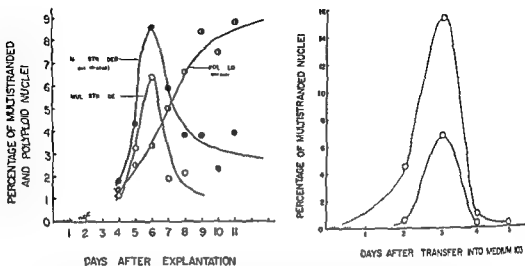


FIG. 161 (*Left*) Frequency of multistranded and polyploid nuclei in unaltered monkey kidney cultures as a function of time (*Right*) Frequency of multistranded nuclei in altered monkey kidney cultures as a function of time (data from two separate experiments)

be related directly to alteration we did observe interesting chromosome irregularities that I shall now describe. Apart from dividing cells with the normal diploid complement of 42 chromosomes a large number of nuclei showed other types of division figures including tetraploid nuclei (one of which is shown in Fig. 160 *bottom left*) and nuclei similar to those observed by Evan and Hauschka in mouse ascites tumors and described by them as endoreduplicated metaphases. Because of the uncertainty as to the origin of these forms in our material we shall refer to them only as multistranded nuclei. A 4 stranded tetraploid is shown in Figure 160 *bottom right*. We have also observed 4 stranded nuclei of even higher ploidy as for example 8 stranded octoploids.

In a series of experiments designed to study the appearance and the fate of these unusual division figures as a function of time freshly isolated kidney tissue was trypsinized and cell suspensions were placed for incubation in 100 petri dishes that were maintained in a controlled atmosphere to prevent evaporation and loss of CO<sub>2</sub>. Each dish contained 2 microscope slides upon which cells would settle and proceed to multiply. In order to have slides for study over an extended period as many as 100 cultures were prepared from a single pooled suspension

and since preliminary studies had indicated that the size of the inoculum might be important, 2 concentrations of cells were used that differed by a factor of 4. The medium consisted either of CMRL-1066 supplemented with 20 per cent horse serum or one comprised of calf serum, lactalbumin hydrolysate, yeast extract, Tris buffer and inorganic salts which we shall refer to as medium 103. On the second day the cultures were washed and the medium renewed thereafter the medium was renewed daily. On the third day and at daily intervals until the end of the experiment slides were removed from the dishes, treated with a hypotonic saline solution for 30 minutes to spread the chromosomes, fixed for 5 minutes in acetic alcohol, dried in air and stained with aceto-orcein. The preparations could be studied as temporary mounts and if necessary could be made permanent by the CO<sub>2</sub> ice method of Conger and Fairchild. For each slide that was scored about 1000 cells in metaphase were completely classified as to ploidy and strandedness. This classification was not based on detailed chromosome counts in all cases. Thus for example if the chromosome mass suggested 42 chromosomes the nucleus was scored as diploid; if the mass seemed twice the size it was scored as tetraploid.

The results of a typical experiment of this

sort are shown in Figure 161 left. It will be seen that both the dilute and the concentrated cell suspensions yielded cultures in which the percentage of multistranded nuclei was low at first but rose rapidly to a peak at about 6 days. In some experiments the percentage of multistranded forms reached values of up to 20 per cent. Beyond 6 to 7 days the percentage of multistranded nuclei decreased and remained low for the duration of the experiment but the highest percentage of multistranded nuclei was always found in cultures initiated from the more concentrated cell suspensions. During the early days the percentage of polyploids always lagged behind that of the 4 stranded forms but increased later to about 10 per cent of the metaphase nuclei. Because these data are preliminary it is perhaps premature to draw definite conclusions as to the induction and the disappearance of multistranded nuclei in cultures of unaltered monkey kidney epithelium. At least 2 possibilities come to mind namely that certain cells of the intact kidney may be predisposed to this condition and cultivation *in vitro* allows the character to be expressed or that the shock of explantation and subsequent cultivation may in itself induce the formation of multistranded nuclei in a certain percentage of the dividing cells.

I shall now describe similar results that were obtained in experiments with altered monkey kidney cells for they clarify to some extent the interpretation of the earlier experiments. It was first found that altered monkey kidney cells under certain conditions also yielded multistranded nuclei an example of which is shown in Figure 167. In experiments designed to study more carefully the conditions under which multistranded nuclei could be obtained the altered cells were propagated in suspension in roller tubes in medium CMPL-1066 supplemented with 20 per cent horse serum. From these suspensions petri dish cultures were prepared as before in another medium No. 103 and colchicine was added about 18 hours before the slides were removed for staining. As in the earlier experiments a daily estimate of the percentage of multistranded nuclei was made from the third day. In Figure 161 right which includes data from 2 experiments the results are strikingly similar to those obtained with unaltered cells except that the highest percentage of multistranded forms occurred somewhat earlier at 2



FIG. 162 Photomicrograph of a metaphase plate of an altered cell from a clone (27) derived from monkey kidney epithelium. Note the 4 stranded condition of the chromosomes to give twice the basic chromosome number ( $\times 1000$ ).

to 4 days. This effect was found in each of several experiments though the magnitude of the peak values varied and was sometimes as high as 30 per cent. However when cells propagated in suspension in CMPL-1066 supplemented with horse serum were transferred to petri dishes containing the same medium rather than 103 few multistranded nuclei were found. Also when the reverse transfer was made for example when cells propagated in medium 103 were transferred to petri dishes containing supplemented 1066 little or no induction of multistranded nuclei occurred.

These results indicate that it is not the change of medium *per se* that is responsible for the appearance of multistranded nuclei but rather that the initiation of cell division in medium 103 stimulated in some way the appearance of these nuclei. Of course these observations do not suggest that these are the only conditions that might effect their induction. In fact in preliminary experiments we have found that altered cells that were propagated in petri dishes in 103 for more than 5 days then trypsinized and replated in 103 also yielded elevated percentages of multistranded nuclei. Similar results have been observed occasionally when unaltered cells grown in petri dishes in 103 were trypsinized and replated in 103. Also large percentages



of multistranded nuclei have occasionally been found even without medium 103 in cultures in which unaltered cells were placed immediately after explantation in supplemented 1066.

The experiments with altered cells would seem to indicate that the induction of multistranded nuclei can occur in the absence of genetic predisposition since the altered cells used for these experiments were derived by single-cell isolation. It would seem more plausible to suggest that the multistranded forms arise as the result of some sort of shock, both in unaltered and altered cell material.

Comparison of the rates of formation of multistranded and polyploid nuclei in the unaltered cell cultures indicates that the former appear before the latter and that as time goes on the multistranded forms tend to disappear whereas the polyploids remain. Although the extent of polyploidy was not scored carefully in the experiments with altered cells, cultures that had previously shown a large percentage of multistranded nuclei later showed a large percentage of polyploids. These results show quite clearly then that in our material polyploid forms are derived from multistranded nuclei. To this extent our results support the similar conclusion of Levan and Hauschka.

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TABLE 107 ANAEROBIC GLYCOLYSIS IN NORMAL MALIGNANT AND TRANSFORMED CELL LINES IN TISSUE CULTURE

		NORMAL (FIBROBLASTS)	MALIGNANT (HEP No 2)	TRANSFORMED (CHANG'S LINES)
N Q <sup>14</sup> CO <sub>2</sub>	Exp 1	16	54	40
	Exp 2	9	37	69

Q =  $\mu$ l of CO<sub>2</sub>/hr/mg dry weight, or c.f.f. CO<sub>2</sub> tension in the culture media chamber

Leslie Fulton and Sinclair in a metabolic study of an altered cell line derived from normal human embryonic livers found that the glucose consumption of their 3 morphologically normal cell lines recently isolated from embryonic skin, kidney and lung were closely similar whereas their morphologically different cell line resembled that of the 3 human carcinomas studied. Metabolic studies along these lines are just beginning and high hopes may be entertained for them to be of considerable help in following the change from normal to malignant.

**Nutrition.** If normal and known cancer cell lines had different nutritional requirements it might be possible to use this as a criterion for malignancy. Although cells kept in continuous culture are known to change their nutritional habits as yet it is impossible to distinguish them on this basis. Chang noted that during the isolation of conjunctival, liver, kidney and appendiceal cell lines from normal individuals their sensitivity to human sera was quite different. Later this sensitivity disappeared. Puck and his group in studies already mentioned found that nutritional requirements of the Chang cell lines were identical to the S3 line of HeLa cells. On the other hand Parker has noted that monkey kidney cultures kept in chemically defined media without a protein supplement have failed to show altered cells in 3 months and that the cells remained in good condition. This finding may be a hint on how to keep our normal cells normal.

#### CHANGES IN REACTIVITY

When a cell line changes in morphology one might expect it to change in either its nutritional or biochemical requirements or in its viral susceptibilities. Eagle has pointed out that a strain of HeLa which assumed a fibroblastic

form concomitantly showed a biochemical alteration. A loss in cytopathogenicity of polio virus for altered cell lines has been reported by numerous workers. In addition Bang and Gey had observed that Eastern equine encephalitis selectively destroyed the cells of a derived rat sarcoma their T 333 and had no effect whatever on the parent fibroblast. However due to the great variety of cytopathogenic effects that one observes when a number of viruses are screened against a number of cell lines it seems unlikely that this method could be used to determine whether a given cell line is cancerous or not. Of course if a cell line becomes resistant or susceptible to a certain virus or chemical one could use this as an indication that a change of some sort had taken place.

#### TRANSPLANTATION

The ability of cultured cells to produce tumors in the homologous strain of animal has long been a criterion for neoplasia. In the animal studies with rodent fibroblasts which already have been cited cell lines became capable of producing tumors which on histologic examination were sarcomas and could be transferred to other animals of the same strain indefinitely. It is impossible to study human altered cell lines in exactly this same way but they can be tested for their ability to produce tumors by implantations into cortisone treated and irradiated weanling rats and also into volunteer cancer patients and normal volunteers. Before presenting the results it is necessary to discuss what is meant by a positive or a negative result and here of course there is considerable disagreement. It seems that there is no absolute criterion for calling a tumor a cancer although the diagnosis is made every day. The pathologist makes up his mind on the basis of clinical findings, cell

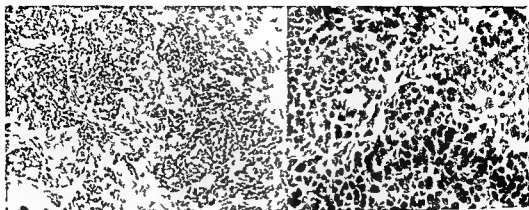


FIG. 166 (Left) Tumor produced by the Chang conjunctival cells 53 days after the first biopsy and 65 days after inoculation ( $\times 46$ ) (Right) Same section ( $\times 145$ )

appearance and a vast amount of experience. Sections of the tumors produced by our inoculations of the Chang cell lines were reviewed by 4 pathologists and were found to contain cells which looked like epithelial cells with certain characteristics that are associated with neoplasia and if they had been submitted for diagnosis would have been called cancer. It immediately is recognized that this is insufficient evidence for calling these particular cell lines cancerous because cancer is regarded as a progressive growth which leads to the death of the host animal and this is certainly not the case here. Keeping this in mind a positive result is defined as the appearance at the site of tumor implantation of a nodule 7 to 14 days postinoculation with no sign of a nodule during the intervening time which when removed by biopsy shows epithelial cells in an array and of a type suggesting neoplastic growth.

Very briefly in the animal transplantation studies cited here the Chang cell lines were inoculated in quantities of 1 to 4 million subcutaneously into the irradiated and cortisone treated rats in 25 instances and tumors were produced in 13 which contained epithelial cells and were considered to be positive. Known cancer cell lines almost invariably produced tumors so there is no doubt that the Chang cell lines are much less vigorous in this regard. Control inoculations of cell suspensions of human testicle (4 attempts), placenta (2 attempts) and monkey kidney (1 attempt) failed to produce tumors as did a variety of tissue minces from normal lung and breast. This is in

contrast with the experiences of Dr. Coriell who obtained the same type and number of tumors from monkey and human kidney cell suspensions as he did from the Chang cell lines. He pointed out that these all regress in contrast with HeLa, the only human cell line studied. In turn his findings differ from those of Dr. Ginsberg who studied lines of cells derived from normal human tonsillar and adenoidal epithelium which in quite a short period of cultivation (about 6 weeks) showed an alteration. When inoculated into irradiated and cortisone treated rats 85 to 100 per cent developed tumors which invaded muscle and submaxillary gland and by these criteria were much more malignant than the HeLa strain which he used as a control.

Probably there are adequate explanations for difference in results obtained. For example as Ginsberg pointed out his strain of HeLa might not have been as invasive as that of Coriell and it may be that the normal cell suspensions with which we failed to produce tumors were not of good enough quality or were in insufficient numbers.

We have also used human volunteers both with and without cancer for implantation experiments by inoculation of 1 to 5 million cells subcutaneously into the flexor surface of the forearm where no lesions were present. Tattoo marks were placed directly over the implantation site and excisional biopsies were done whenever nodules become palpable—usually 9 to 14 days after inoculation. Two types of controls were used for these experiments: human

embryonic and adult fibroblasts which had shown no morphologic change since they were isolated and known cancer cell lines. In the cancer patients (Table 108) 13 implantations of Chang's cells were made. 7 were biopsied and contained epithelial cells and were considered as positive according to our definition. 3 had no palpable nodules and therefore were considered clear-cut negatives and 3 had nodules which regressed. Nineteen cancer patients were inoculated with cancer cell lines. 15 biopsies were done and 14 contained cancer cells. In 2 nothing could be palpated so no biopsy was done and in 2 instances the nodules were allowed to regress. When ever possible the biopsy sites were rebiopsied at postmortem and in 1 instance of 3 with the Chang cells and 7 of the 3 with the human cancer cell lines tumor cells were found. Figure 166 left shows the histologic appearance of the Chang conjunctival cell line 53 days after the first biopsy and 63 days after implantation. The cells are grouped in columns and cords embedded in connective tissue. Higher power (Fig 166 right) shows that the cells are epithelial in type with very little sign of degeneration. No tumors were formed from the 3 human fibroblast implants. It should be pointed out that there is a great amount of individual variation in the cancer volunteer's resistance to inoculation of either the Chang cell lines or the known cancer lines. For instance both positive rebiopsies of the tumor cell lines occurred in 1 patient whose susceptibility to cultured cells is attested to by the fact that all implants have taken and at autopsy a metastatic node was found from a chorio-allantoic membrane grown HEP No. 3.

TABLE 108 RESULTS OF INOCULATIONS OF CELL LINES INTO VOLUNTEER CANCER PATIENTS

	CHANG CELL LINES	CANCER CELL LINES
Number of implants	13	19
Positive biopsies	7/7†	14/15‡
Nothing to biopsy	3	2
Nodules which regressed	3	2
Positive rebiopsies	1/3†	2/3‡

† Local sites 14/14 free of implants  
 ‡ Sites at the number of implants  
 b p e s p e r f m t

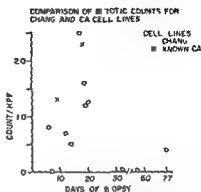


FIG. 167 Average number of mitotic figures per high power field (average of 70) of the tumors produced by known cancer cell lines and the Chang cell lines.

Unfortunately one cannot compare directly the malignancy of the Chang cell lines and the known tumor cell lines. Although it is possible to note such things as degenerating cells, host reaction and so forth, all of these measurements are subject to error. One might prognosticate that one tumor would regress or continue to grow but there is no way of proving it. It is possible to obtain a general idea of the activity of growth by doing mitotic counts and this was done for both groups of tissue by noting the number of mitotic figures seen in an average of 70 fields containing good tumor tissue. Figure 167 shows that there is great variety in the number of mitotic figures in both types of tissue, some of Chang's cell lines having as high an index as the known cancer cell lines and some of the latter being singularly lacking in mitotic activity.

In the normal human volunteers receiving known cancer cell lines 4 of 10 implantation sites were found to contain cancer cell lines on biopsy 14 days later. However I do want to emphasize that these cells which were found were quite different from those that we find in the cancer patients in that there was a lot of reaction and the cells were mostly degenerating. No tumors formed in these normal volunteers when the Chang cell lines (3 implants), the HeLa cell lines (2 implants) or human fibroblasts (3 implants) were inoculated.

In addition to the Chang cell lines we have done some limited studies on the H strain of

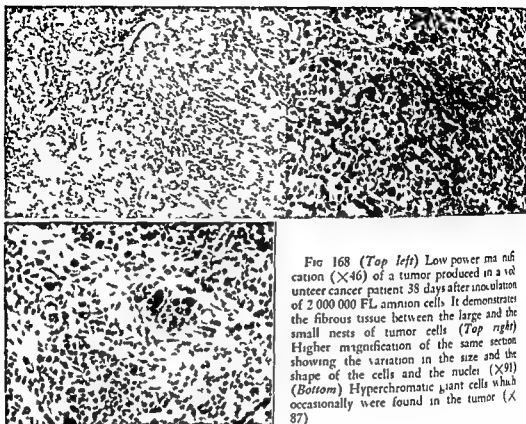


FIG 168 (Top left) Low power magnification ( $\times 46$ ) of a tumor produced in a volunteer cancer patient 38 days after inoculation of 2 000 000 FL amnion cells. It demonstrates the fibrous tissue between the large and the small nests of tumor cells (Top right) Higher magnification of the same section showing the variation in the size and the shape of the cells and the nuclei ( $\times 91$ ) (Bottom) Hyperchromatic giant cells which occasionally were found in the tumor ( $\times 87$ )

amnion which was obtained through the courtesy of Dr Jorgen Fjell. This cell strain originally derived from human amnion had had 40 trypsinizations when we received it. Two million cells were implanted into 2 sites in the forearm of a volunteer patient who had reticulum cell sarcoma. A tumor appeared in both sites. 1 was biopsied 14 days later while the other was allowed to remain in situ where it continued to grow slowly until the death of the patient from his own disease 38 days later. Figure 168 shows the microscopic appearance of the tumors. Dr Stephen S Sternberg who examined these sections gave the following report:

Sections reveal tissue having all the morphological criteria of a malignant tumor. The growth pattern shows considerable variation in different areas. A major portion of the tumor is growing in solid masses; in some portions in small nests. Both connective tissue and adipose tissue are involved. The cells show moderate variation in size and shape. There is nuclear irregularity and hyperchromatism. Many cells

have enlarged dark and prominent nuclei. The cytoplasm is abundant and generally pale and either homogeneous or granular. In some cells the cytoplasm is vacuolated and in others mucus like secretions appear to be present. Occasional tumor giant cells are present.

Chromosome studies were also conducted. Dr A Levan and Dr J Bieseke who found the chromosome number varied around 80 and that many chromosomes were abnormal.

## DISCUSSION

It is apparent that we do have methods and techniques by which cells grown in tissue culture can be characterized. It seems clear that judgment of the cell status cannot be made on one test alone but if in a number of tests a cell line seems to resemble the malignant prototype more than the normal one is justified in doubting its claim to normality. This does not mean that it can be said to have become malignant even though it is capable of producing tumors which ordinarily would be termed malignant.

since the clinical criteria of lethality and metastases have not been fulfilled.

The point which I believe needs emphasis is that cells derived from normal sources and maintained in tissue culture should be watched constantly and their characteristics determined so that when they are referred to as normal we may have some assurance that they do have the morphological, metabolic and growth characteristics that we associate with normal cells.

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## DISCUSSION

DR SYVERTON Drs Eagle Puck Parker and Moore in these 4 excellent papers have exemplified the state of sophistication reached by animal cell culture. Time is inadequate to consider implications of these papers save for one point. The conjunction of subject matter perhaps inadvertent emphasizes a problem facing all who work with established strains of animal cells in virology. Several years ago we foresaw need for mammalian cell strains in continuous culture for poliomyelitis research. The cells developed were obviously potentially valuable for vaccine production but at first the cell strains were of malignant origin. So there appeared what Westwood and his associates aptly termed the "specter of malignancy of cultivated cells." Specters are notoriously tenuous and pervasive. This one no exception has gotten mixed up with most of the changes we see in mammalian cells as part of the normal spectrum during serial cultivation. The 4 papers indicate dimensions of the problem and suggest means of attack.

I suggest that we as virologists keep our objectives firmly in mind. The cancer researcher is interested in cell transformations pertaining to malignancy; as virologists we are interested in cell transformations pertaining to normality. If fear of malignancy should prevent application of advanced cell technique to preparation of vaccines protective against old and new virus diseases that will be a consequence serious enough failure to understand the bases of departure from normality on the part of cultivated cells will be tragic since it will prevent us from translating cell-culture studies into terms of disease in vivo.

I believe that these dangers can be avoided if we will concentrate on investigation rather than speculation. It is true as Earle Grey and others have shown that malignant transformation is a possible rare and most extraordinary hazard that may occur during continuous cultivation of mammalian cells. It is not known that such transformation is an inescapable hazard or even that it occurs from other than extrinsic cause. Altered cells are a legitimate and fascinating objective study for both virologists and cancer

biologists. As an approach to this problem a broad program aimed at the derivation of new cell lines and the characterization of the new strains of both normal and malignant cells has been instituted in our laboratories. These criteria are shown in Table 109. Elsewhere we emphasized the importance of rigidly standardized technique for propagation of cells used in virus research and described methods for concomitant study of the growth and the metabolism of mammalian cells. Such broad characterization of cultivated cells as indicated in this table must be applied in conjunction with search for means of establishing human cells in continuous culture under known environmental chemical and physical conditions calculated to preserve normality and not to induce abnormality.

The points brought out in this table include origin. It is absolutely imperative that the species origin of each cell line employed be established whether derived from man, mouse or other animal. This is part of the identification.

The Growth Phase lag rate duration of unfed cultures. These lines vary remarkably.

TABLE 109 COMPARATIVE PROPERTIES OF HUMAN CELLS IN CONTINUOUS CULTURE

Origin	confirmation as human and not other species
Growth phase	lag rate duration unfed cultures
Size	volume and extension
Metabolism	a ratio of catabolism to respiration b oxygen consumption ( $Q_{O_2}$ ) c evidence for inefficient or uncoupled respiration d essential nutritional elements
Transformation	ability to transform and monkey normal and pretreated with cortisone and $\gamma$ irradiation
Infectiousness	response to viruses of T13 poliomyelitis vaccine and herpes
Potential	irradiation dose required for (a) destruction (b) inhibition of capacity to produce virus (c) inhibition of capacity to infect

*Size* volume and extension

*Metabolism* a battery of tests of metabolism are applied as routine

*Transplantability* to hamsters and monkeys normal and pretreated with cortisone and x irradiation A cancer patient is a sick patient

*Virus Spectrum* response to a battery of viruses and not to a single virus which of itself may well be altered

*Response to Irradiation* as outlined by Dr Puck in part

More attention must be paid to the potentialities of the normal cell. Otherwise we should have to dismiss all cells in culture inclusive of monkey kidney cells since they grow as cells in vivo do not. Methods and results discussed by Drs Eagle and Puck can be extended (1) to indicate in terms of plating efficiency the suitability of defined media for optimum propagation of cells direct from normal tissue (2) to define nutritional requirements of such normal cells and (3) to supplement defined media so as to permit propagation of particular and the more fastidious cells. Finally we need improved methods for differentiation and identification of cell types at both species and cellular levels. The characteristics of each cell line must be established. Meanwhile to reveal the nature and the mechanism of these intriguing alterations of cells so that the changes can be avoided or produced at will such work as we have heard reported by Dr Parker must continue.

As a discussant I believe that we have been most fortunate in having this excellent group of papers presented today.

**DR DULBECCO** We discuss some of our recent results on the nature of variants of HeLa cells which can be obtained by exposing the cells to poliomyelitis virus. These results also have some bearing on the problem of virogeny in these cells.

The variants were isolated by following the procedure of Ackermann and Kurtz. Cells of the S3 strain were infected at a multiplicity less than 1 with an attenuated strain of polio virus Type 3 kindly supplied by A H Sabin. The infected cells were then cultivated in a medium containing antiviral serum. The progeny of the surviving cells was exposed again to virus in a similar way. Five serial steps were carried out all similar except for the multiplicity of the virus

which was steadily increased to reach the value of about 8 p.f.u. per cell in the last step.

The cell population obtained after this procedure is called the resistant culture. It was composed by elongated fusiform cells different from those of the usual S3 type in agreement with the previous findings of Kurtz.

This resistant culture was investigated in several ways. A first series of experiments concerned the fate of the culture in subsequent mass transfers. At every transfer 2 cultures were set up: 1 with and 1 without antiviral serum. For 8 serial passages the culture without antiviral serum degenerated after a few days; abundant virus could be found in the supernatant. The cultures containing antiserum did not degenerate when their cells were trypsinized and plated on monolayers of monkey kidney cells for viral plaques: a proportion of them was found to be virus producing in the early passages. This proportion was 5 per cent at the second passage and 1/10 000 at the third passage. This proportion was not significantly different when determined on monolayers of giant HeLa cells obtained after x ray treatment.

At the seventh passage the cells of the culture with antiserum no longer produced plaques on monkey kidney cells; the culture without antiserum did not degenerate. In subsequent passages without antiserum the progeny of this culture did not degenerate any longer and no production of virus could be detected. These results show that the cells of the original resistant culture were not permanently avirulent although the cultures kept with antiviral serum displayed what appeared to be a transient virogenicity. This apparent virogenicity may well be due to a complex dynamics of cellular infection by virus and of virus inactivation by antiserum and may be therefore of trivial nature. The nature of the apparent virogenicity was studied further by experiments that gave the results to be described presently.

It was found that the fusiform cells present in the resistant culture are not necessarily associated with virogenicity for two reasons. The first reason is that nonvirogenic population derived from the seventh passage of the resistant culture had the same fusiform cell type as the apparently virogenic cultures of earlier passages. The second reason is that cells of the fusiform type can be found with a frequency of a few

per cent in the untreated S3 culture as shown by examining unselected clones

To clarify further the nature of the cells present in the resistant culture pure lines were obtained from its seventh avirulent passage by an improved technique which allows the isolation of cell populations derived with certainty from a single cell. This technique is a development of the single-cell technique described by Lwoff *et al.*—individual cells obtained by trypsinization were transferred under the microscope to large drops of medium kept under a thick layer of paraffin oil. The individual cells started to multiply after a lag period of less than 24 hours and divided regularly with a constant doubling time of 70 hours to give rise to large populations. Among the pure clones observed 3 different morphologic types were observed: 1 very elongated called F (fusiform), 1 less elongated called I (intermediate) characterized by a typical pattern of the clones and 1 polygonal called P. Cells of F and I type were main components of the seventh passage culture cells of P type were rare.

The virus resistance of these 3 pure cell lines was investigated by determining the proportion of cells able to produce a clone as a function of the multiplicity of virus infection. I and F cells were found to be more resistant than S3 cells whereas the resistance of P cells was almost identical to that of S3 cells. The nature of the resistance was brought to light by the survival curves. These showed that absolutely resistant cells were absent, the increased virus resistance of the F and I line could be attributed to a decreased probability that a cell became infected when a virus particle became attached to its surface. Other experiments showed that once the resistant cells became infected they reproduced the virus like the more sensitive cells. The varying slope of the two curves shows that both populations obtained cells of different resistance although the F population was much more homogeneous than the S3 population. Both populations contained a fraction of highly resistant cells (lesser slope) for which the probability of infection was about 1/70 of that of the most sensitive cells contained in the S3 culture. The S3 culture contained about 5 per cent of the highly resistant cells, the F culture contained about 50 per cent of them. In addition the F culture did not contain any important fraction

of cells of high sensitivity. It is interesting that the proportion of highly resistant cells present in the S3 culture was of the same order as the proportion of the fusiform cells found in non-infected S3 cultures.

The phenomenon of temporary virulence of the resistant cultures at the early passages was investigated further by studying the fate of individual infected F cells. In F culture was infected and the infected cells were trypsinized and transferred individually to drops of fluid under paraffin oil in the presence of antiviral serum. The cells were examined daily and the medium was assayed periodically for presence of virus. About 200 cells were studied. Of these about 75 per cent degenerated within the first 24 hours virus was found in their supernatants in 80 per cent of the cases. The rest survived and gave rise to clones the supernatant of which was always free of virus. In 3 cases virus was found in a drop containing a cell that had divided however in all 3 cases degenerated cells appeared fairly soon in the drop, none of these cells gave rise to a permanent clone.

The experiments show that the F cells do not become permanently virulent with a frequency greater than 1 per cent. A temporary carrier state lasting not longer than a few divisions seemed on the contrary to occur in about 1 per cent of the cases.

In conclusion the effect of the virus could be shown to consist of a selection for special pre-existing cell types. These cellular types differ in their virus resistance from the majority cell type present in the population before infection; they are different also in other characteristics such as their morphology as has been shown and some physiologic properties which were not discussed. They do not appear to be or to become permanently virulent.

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Dr. Metzger. My remarks are an extension of Dr. Eagle's paper because in New Haven with the development in a synthetic medium of the growth of monkey kidney cells and also the growth of virus in such cells.

TABLE 110 SYNTHETIC MEDIUM (SM 2) FOR GROWTH OF MONKEY KIDNEY CELLS

AMINO ACIDS	MG /LITER	VITAMIN MIXTURE	MG /LITER	BASIC SALT	Gm /LITER
1 isoleucine	300	Folic acid	40.0	NaCl	8.0
1 leucine	200	Biotin	0.15	KCl	0.4
1 lysine	60	Nicotinic acid	0.15	MgCl <sub>2</sub> 6H <sub>2</sub> O	0.1
1 histidine HCl	10	Pyridoxine	0.115	MgSO <sub>4</sub> 7H <sub>2</sub> O	0.1
1 valine	170	Thiamine	0.15	Na HPO <sub>4</sub>	0.06
1 arginine	60	Pantothenate	0.10	NaH <sub>2</sub> PO <sub>4</sub>	0.06
1 phenylalanine	90	Riboflavin	0.15	CaCl <sub>2</sub>	0.4
1 tyrosine	145	Vit B <sub>12</sub>	0.015	NaHCO <sub>3</sub>	See note
1 tryptophan	5	Choline	1.00		
1 methionine	12	Inositol	3.50		
1 threonine	60	Ascorbic acid	50.00		
1 cysteine HCl	100				
glycine	150				
1 glutamine	280				
MISCELLANEOUS	MG /LITER	TRACE ELEMENTS		M x 10 <sup>4</sup> /LITER	
Lecithin	25	Fe (NO <sub>3</sub> ) <sub>3</sub> 9H <sub>2</sub> O		2.0	
Cholesterol	10	CoCl <sub>2</sub> 6H <sub>2</sub> O		0.7	
Ethyl alcohol		MnCl <sub>2</sub> 4H <sub>2</sub> O		0.5	
(a diluent for		ZnSO <sub>4</sub> 7H <sub>2</sub> O		3.0	
lecithin and		(NH <sub>4</sub> ) <sub>2</sub> MoO <sub>4</sub>		1.0	
cholesterol)	590	CuSO <sub>4</sub> 5H <sub>2</sub> O		0.4	
Glucose	1 000				
Pyruvate					
(sodium salt)	500				

Notes on preparation

Amino Acids—held as 20 x concentrated combined stock without tyrosine cysteine glutamine Tyrosine held as 0.2 per cent stock cysteine held as 10 per cent stock at 4 °C glutamine held as 2 per cent stock at -20 °C

Amino acids sterilized by filtration

Vitamin Mixture—held as 100 x conc stock without ascorbic acid Sterilized by filtration

Miscellaneous—Lecithin (Pfanzstiel ex ova) held as 10 per cent stock in absolute ethanol cholesterol as 2 per cent stock in absolute ethyl alcohol

Basic Salt—held as Hanks's salt 10 x conc with glucose supplemented with 0.26 Gm/liter of CaCl<sub>2</sub> and with bicarbonate after the medium has been made up CaCl<sub>2</sub> held as 10 per cent stock NaHCO<sub>3</sub> concentration for outgrowth from trypsinized suspensions 1.1 Gm/liter for replenishment 1.9 Gm/liter

Trace Elements—held as separate stocks 0.05 per cent conc Fe (NO<sub>3</sub>)<sub>3</sub> sterilized by filtration

To prepare 1 liter—add in order to 750 ml distilled demineralized H<sub>2</sub>O

Hanks's 10 x conc with glucose	100
SM 2 Amino acid conc 20 x	50
Tyrosine 0.2 per cent heated to dissolve	70
Glutamine 2 per cent	14
Cysteine 10 per cent	1
Vitamin mixture 100 x conc	10
Ascorbic acid	0.5
Lecithin	0.25
Cholesterol	0.5
NaHCO <sub>3</sub> (7.5% solution)	15-75 (see note above)
CaCl <sub>2</sub>	2.6
ZnSO <sub>4</sub> 7H <sub>2</sub> O	0.2
Fe (NO <sub>3</sub> ) <sub>3</sub> 9H <sub>2</sub> O	0.2
CuSO <sub>4</sub> 5H <sub>2</sub> O	0.05
CoCl <sub>2</sub> 6H <sub>2</sub> O	0.05
MnCl <sub>2</sub> 4H <sub>2</sub> O	0.10
(NH <sub>4</sub> ) <sub>2</sub> MoO <sub>4</sub>	0.2

The synthetic medium which Dr Rappaport has developed is shown in Table 110 and as you can see contains the essential amino acids and also cystine, glutamine and another requirement which Dr Rappaport has found to be essential for monkey kidney cells namely glycine. The notable difference between this medium and those which others have used has been the fact that there is additional calcium in this medium and trace elements but particularly the amounts of calcium used are about 3 to 4 times that commonly used in salt solutions such as Hanks's or Earle's. This particular medium will support the exponential growth of monkey kidney cells for a few weeks with a mean generation time of 24 hours. This curve (Fig. 169) was constructed by determining the cell count daily on a number of cultures by a method that Dr Rappaport has recently developed of measuring decoloration of phenol red 15 seconds after it is added to such cultures. It should be emphasized that the addition of serum to this synthetic medium does not increase either the rate or the duration of cell growth.

Two requirements of the physical environment have been established. As I mentioned a concentration of calcium 3 to 4 times higher than that in Hanks's original formula is necessary for growth. Without this calcium supplement the cells stop spreading, along the edges of the cells become thickened and the cells granulated.

Even more critical than the presence of glycine or the concentration of calcium is the type and conditioning of the glass used for the cultures. The medium SM-7 supports exponential growth on soft glass but will not support even survival for 3 days on hard pyrex glass unless the glass is conditioned by a preliminary rinse with sodium hydroxide. This is in order to replace the surface bound proton with sodium ion.

It has been found that polio virus is propagated in SM-7 monolayer cultures in a salt glucose medium in the absence of any exogenous nitrogen source as well as in the complete medium containing calf serum lactalbumin hydrolysate. The rate and the amount of virus produced during the first 24 hours after the cells are transferred to the salt solution is independent of any exogenous carbon source.

Thus the nitrogen, the organic carbon and

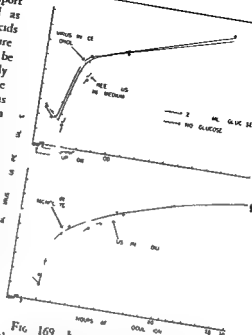


Fig. 169 Kinetics of polio virus synthesis in monkey kidney cells in the presence and the absence of glucose. Nine-day old monolayer cultures of monkey kidney cells washed and then inoculated with  $3 \times 10^4$  plaque forming units per culture with or without glucose in Hanks's salt solution. Cultures washed free of unadsorbed virus and replenished with Hanks's salt solution. Intracellular (plus cell bound) virus and free virus in the medium titrated by plaque technique at times indicated.

all the energy for the synthesis of virus must be derived from the endogenous reserves of the cell. Since the total endogenous polysaccharide of the culture of 1 000 000 monkey kidney cells varies between 30 and 50 gamma equivalents of glucose and since the cultures can utilize 0.00 gamma of glucose per hour it would seem that during the 24 hours it is a very small fraction of the cell's total energy producing capacity.

Only two constituents of SM-7 have been found to affect the course of virus propagation. Typical data for the effect of cystine in this system are given in Table 111. At any given glucose concentration cystine decreases the time when cytopathogenic changes are complete and

TABLE III STIMULATORY EFFECT OF CYSTEINE ON VIRUS SYNTHESIS  
IN M CULTURES IN SALT GLUCOSE SOLUTION

MEDIUM HANKS'S SALT SOLUTION SUPPLEMENTED WITH		TIME OF HARVESTING (WHEN CPE IS COMPLETE) (HRS)	PFU PER CULTURE (LOG)	VIRUS YIELD PER CELL (PFU)
CARBON SOURCE GLUCOSE ( $\mu$ G / 1 L)	NITROGEN SOURCE CYSTEINE ( $\mu$ G / 1 L)			
3.0	—	90	8.8	60
	50	60	9.4	1000
0.9	—	49	9.0	400
	50	43	9.4	1050
0.3	—	49	8.6	0
	50	49	9.0	400
3.0	—	95	8.4	110
	50	55	9.0	400
0.9	—	71	8.7	00
	50	55	9.2	630
0.3	—	71	8.3	7
	50	47	9.1	50

Mixture of 3.0  $\mu$ Cu, 0.9  $\mu$ Cu, 0.3  $\mu$ Cu, 0.1  $\mu$ Cu, 0.05  $\mu$ Cu, 0.025  $\mu$ Cu, 0.0125  $\mu$ Cu, 0.00625  $\mu$ Cu, 0.003125  $\mu$ Cu, 0.0015625  $\mu$ Cu, 0.00078125  $\mu$ Cu, 0.000390625  $\mu$ Cu, 0.0001953125  $\mu$ Cu, 0.00009765625  $\mu$ Cu, 0.000048828125  $\mu$ Cu, 0.0000244140625  $\mu$ Cu, 0.00001220703125  $\mu$ Cu, 0.000006103515625  $\mu$ Cu, 0.0000030517578125  $\mu$ Cu, 0.00000152587890625  $\mu$ Cu, 0.000000762939453125  $\mu$ Cu, 0.0000003814697265625  $\mu$ Cu, 0.00000019073486328125  $\mu$ Cu, 0.000000095367431640625  $\mu$ Cu, 0.0000000476837158203125  $\mu$ Cu, 0.00000002384185791015625  $\mu$ Cu, 0.000000011920928955078125  $\mu$ Cu, 0.0000000059604644775390625  $\mu$ Cu, 0.00000000298023223876953125  $\mu$ Cu, 0.000000001490116119384765625  $\mu$ Cu, 0.0000000007450580596923828125  $\mu$ Cu, 0.00000000037252902984619140625  $\mu$ Cu, 0.000000000186264514923095703125  $\mu$ Cu, 0.0000000000931322574615478515625  $\mu$ Cu, 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increases the yield of plaque forming units per cell. If the yields obtained in the presence of cystine are corrected for thermo inactivation of virus it is seen that monkey kidney cells can produce at least 2 000 active virus particles per cell.

As already mentioned glycine is required for the growth of monkey kidney cells. However glycine inhibits the synthesis of polio virus. Figure 170 shows that in the presence of glycine plaque numbers under a protein free synthetic medium used in the agar overlay are reduced and the rate of growth of the plaques is retarded. Varying concentrations are shown in the bottles; the control is on the left and the falling concentrations go from left to right.

The results in Figure 171 show that there is a 2 to 3 log inhibition of polio virus synthesis during the first 40 hours in the medium containing glycine. A replenishment of this medium and thus of the glycine every 10 hours prolongs the inhibition of virus growth.

It appears from these studies then that the synthesis of polio virus by monkey kidney cells is not an invariant consequence of the absorption of the virus to the cell but actually may be controlled by specific transients in metabolism. These transients (ordinary amino acids like cystine and glycine) may be favorable or unfavorable to virus synthesis and they may be induced by compounds which are physiologically for the cell.

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DR LLOYD. All those who have had animal cell cultures for some time have noticed morphological or physiologic changes in them. Therefore it was important to find out whether these changes come from a selection of a cellular type pre-existent in the culture or from a spontaneous or induced change.

In order to solve that problem it was important to develop a technique which would facilitate the isolation of clones in other words populations emanating from a single cell. The first clones were obtained by the Wilton Earle group. The technique was extremely elegant but some what lengthy and delicate. Thanks to the medium defined by Theodore Puck it is possible

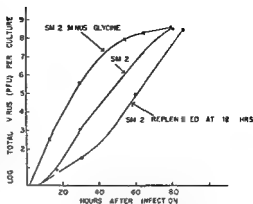


FIG 171 Effect of glycine on propagation of Type 1 polio virus using complete growth medium SM 2 and SM 2 minus glycine. Ten-day-old 2-oz bottle cultures grown in SM 2 were inoculated with 5 10 PFU. After 1 hour at 37° C cultures were washed and replenished with indicated medium. There was a 2 to 3 log inhibition of polio virus synthesis during the first 40 hours in the medium containing glycine.

today to isolate clones with the greatest of ease. According to Puck one can obtain clones by fertilizing isolated cells in petri plates and by isolating colonies developed or emanating from a single cell.

Also it is possible to follow a safer technique developed by Raymond Parker of taking a single cell with a micropipette and injecting it into a droplet of a medium under a paraffin slide. You then can take the clone and fertilize it in a bottle. There is also a simpler technique—one used in the Pasteur Institute—that of isolating a single cell with a micropipette and injecting it directly into a tube.

Therefore it is possible nowadays to obtain clones thanks to a great variety of techniques. That is why physiologists and geneticists can tackle a number of basic problems affecting animal cells. One of these is the problem of the origin or the nature of the cell variations which have been observed *in vitro*.

These are not necessarily—as was shown by Raymond Parker—the product of a selection of variations existing in the cells. Rather these variants are the result of the change affecting a single cell—a change, a modification which can



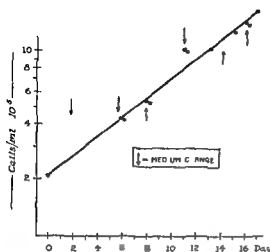


FIG. 172 Growth of L cell in spinner culture

take place a few days after this *in vitro* culture.

The monkey kidney cell then is linked to the number of chromosomes. What is noteworthy is that such modifications seem to take place under the influence of the change of the medium. This is according to Raymond Parker's hypothesis of inducted multiplication coming from the medium—medium induced changes. The understanding of this is particularly important for virologists because we did know as a matter of fact that the variants of the cells which we have gotten *in vitro* can go from sensitivity to the virus to sheer resistance and we also have found that such modifications can bring about changes in the sensitivity to diphtheric toxin. The *in vitro* cell studies then will shed a great deal of light on a number of points which are still much in the dark as far as cell pathology is concerned.

Therefore I would refer to a single point raised by Harry Eagle. He said that the mammal needs 8 amino acids and that the mammalian cells need 13 of them as they are studied *in vitro*. We know that for the syntheses of the amino acids a number of enzymes are necessary. The synthesis of a given enzyme is governed by a single gene and depends on the specific inducer. This specific inducer is exogenous for the nonconstitutive enzymes but they are adaptive or constitutive enzymes for synthesis a specific inducer is needed in either case.

A micro-organism as a whole is a well organized totality. The mammalian cell is an inte-

gral part of a general whole and we know that the functioning of a mammalian cell will depend on the other cells on substances such as hormones which are produced by the other cells of the organism. What is true of the hormones could well be true for these inducers. For example during the development of the cell differentiation could well be linked to modification of the synthesis potency of the specific inducers. The liver cells for example could yield a specific inducer for the syntheses of given enzymes which would act as an inducer for the kidney cells. *In vivo* kidney cells would receive this inducer through the blood whereas the kidney cell preserved *in vitro* would be without this inducer and therefore would not synthesize a specific enzyme. This is only a hypothesis but the fact that we can talk about such a premise and the fact that its importance is worthy of or warrants a study shows that the culture of tissues up to now enables us to tackle a number of fundamental basic problems in the field of physiology and cell biology.

The results which have been obtained by Harry Eagle by Puck by Parker by Alton Moore which have been presented at this meeting are particularly important.

Dr. McLIMANS: The important papers presented have been based primarily on the use of glass monolayer tissue cultures a technique that appears to be ideally suited for certain types of investigations but is not without limitations in some other aspects particularly in securing sufficient quantities of cells for the production of viral antigens for biochemical studies or physiologic investigations.

The submerged culture technique was originally introduced through the imaginative efforts of our moderator Dr. Wilton Earle utilizing a shake flask system. I sincerely hope that he will be able to comment on some of his own observations in this field.

Investigations conducted in our laboratories through the collaborative efforts of my colleagues Drs. Davis, Ziegler, Thomas, Kucera and Larson have established the feasibility of obtaining continuous logarithmic growth of stable mammalian tissue cell lines (Fig. 172) as discrete units (Fig. 173) in agitated fluid suspension referred to by us as submerged culture in volumes of 25 ml. to 5 gallons and cell con-

centrations of 1 to 5 million cells per ml for continuous periods of time up to 5 months. Additionally it has been demonstrated that polio virus proliferates in submerged culture cells (strain HeLa) to high titer exhibiting a more rapid rate of absorption and release of the virus with earlier appearance of cytopathologic changes than in the instance of the same host cell grown as a monolayer on glass.

The use of the submerged culture process for the production of viral antigens certainly possesses great advantages over conventional techniques. However, since a stable host cell line of human or monkey origin must be used for propagation of polio virus and since an adequate criterion of malignancy for mammalian cells has not been established, the fear of transmission of a malignant inducing principle precludes the use of such a system for the production of agents designed for parenteral use in man. A proposed solution to this problem in the case of polio virus would be the establishment in submerged culture of a stable cell line sufficiently far removed from man specieswise as to obliterate the fear of use of such a viral vaccine on the grounds of associated stigma of malignancy.

The tremendously important discovery by Westbrook, Sheffield Smith *et al* that a stable rabbit kidney epithelial line (called ERK or subline KD) would support the propagation of all 3 types of polio virus prompted us to investigate this cell line in submerged culture. We have confirmed their observations in that in monolayer cultures polio virus Types 1, 2, and 3 produce typical cytopathology although to low titer on the first passage in the KD line. On serial passage of the virus strains adaptation took place to the extent of  $10^4$  to  $10^{15}$  titers. Submerged culture studies were then promptly initiated with this cell line and the following results achieved:

Sustained logarithmic growth of the KD cell as discrete units in a spinner culture (100 ml volume) (Fig. 174) in the New Brunswick Fermentor (3 liter volume) (Fig. 175) and in the stainless steel water-jacketed impeller agitator fermentor (5 gallon volume) (Figs. 176 and 177) was readily achieved employing Eagle's medium with the daily addition of arginine and inositol. It should be noted that on the basis of past experience in the antibiotic



FIG. 173. L cell (Earle) grown in submerged culture. Delamater's stain ( $\times 800$ ).

industry, one could predict from the results obtained in a 5 gallon fermentor the success of a venture in a much larger unit.

Growth response curves for the propagation of Type 3 polio virus in serum free Eagle's medium using 4 indicator cell systems—the KD cell, the HeLa, the monkey testicular cell line and the monkey kidney line—were obtained from the 5 gallon fermentor as shown in Table 117. Initial viral inoculum was adjusted to approximately 1 tissue-culture infectious dose per 2 to 4,000 cells. Infectious titers as illustrated of  $10^4$  and  $10^5$  or better at some points were obtained in less than 40 hours. The host cell response to infection was characterized by a rapid drop in viability—viability as loosely judged by staining with trypan blue—but not a drop in total cell count and from a cytological viewpoint the formation of a perinuclear mass indistinguishable from that observed with HeLa.

Since the frequent medium changes—3-day

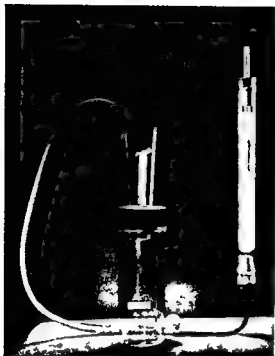


FIG 174 The spinner vessel

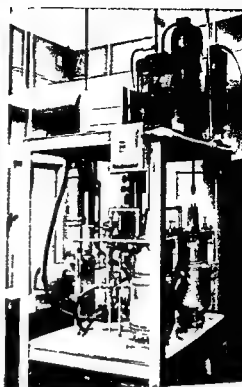


FIG 176 The 20-liter fermentor

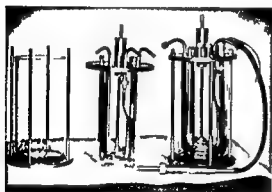


FIG 175 New Brunswick fermentor (NBF) Assembly

to 3-day intervals—become laborious and impractical from the viewpoint of serum requirements particularly with large volumes a search was initiated to find either a substitute for serum or a method of circumventing the necessity for making frequent medium changes. It has been observed in our laboratories that the daily or bidaily addition of arginine permits the maintenance of cell growth in fluid suspension without medium changes for the period of 10 to 30

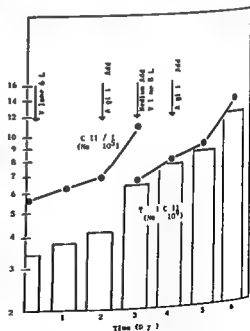


FIG 177 The growth of strain KD cells in the 20 liter fermentor

TABLE 112 HOST CELL (STRAIN ERK/KD)/POLIO VIRUS (T<sub>3</sub>AIM)  
INTERACTION IN 70 LITER FERMENTOR

TIME (HRS)	CELLS PER ML. $\times 10^4$		VIABILITY (%)		TCID <sub>50</sub> /ML	
	CONTROL	INFECTED	CONTROL	INFECTED	MA	MT
0	45	45				
3	47	44	82			
6	47	44	73	87	2.5	3.0
12	43	44	78	78	3.0	2.8
21	50	52	87	81	4.5	5.0
24	52	48	80	80	8.0	6.0
27	61	46	80	80	8.0	5.8
33	67	59	79	40	7.0	6.8
39	65	61	78	31	7.5	6.8
		65	81	15	6.0	7.5
				10	6.5	7.5

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
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1	2	3	4	5	6	7	8	9	10	11	1																																																																																								

days Figure 1/8 demonstrates the role of arginine at various levels in maintaining cell growth as expressed in terms of per cent over the control.

In summary the poliomyelitis vaccine as currently produced utilizing primary monkey kidney cell cultures propagated as monolayers on glass surfaces is costly creates problems relating to contaminants raises questions as to the maximum antigenic mass that could optimally be achieved and is to be sure subject to the ready availability of a constant supply of monkeys. It is hoped that eventually viral antigens will be produced in such a manner as to provide the maximum number of immunizing doses at the minimum cost for all men. In short can we take the monkey business out of the polio vaccine production?

briefly the disagreements to illustrate a few interesting points

On the salt requirements of the HeLa cell Dr Eagle showed that potassium sodium magnesium calcium and phosphate were and that bicarbonate was not essential for cell multiplication and survival. Through the use of a carbon dioxide trapping device Dr Geyer and I showed that bicarbonate was also an essential for cell propagation. We have found that the ordinary medium without the addition of bicarbonate contained about half a millimol presumably through absorption from the air. Through the use of this alkaline trapping device the bicar

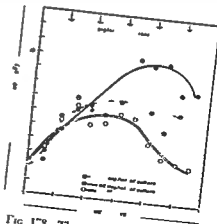


FIG 1-8 The effect of arginine on the proliferation of I cells in submerged culture

bonate concentration was reduced to below the limits of the method of Van Slyke and Neill. While it is true that the HeLa cell would multiply without the addition of bicarbonate they invariably undergo slow degeneration when the absorbed bicarbonate has been removed. I think this illustrates an important point through the use of a more refined technique another ion has been shown to be essential.

The necessity of incorporating whole or dialyzed serum into chemically defined mediums in order to obtain satisfactory growth poses a very great stumbling block in the study of the nutrition of the human cells. Dialyzed serum is a mixture of many many components one or more of which are essential for cell growth. Different sera are known to differ in their growth sustaining properties and I have presented this in a previous conference. I would add one more important difference between the sera—their enzyme activities. We have found that cell multiplication would occur with sucrose as the sole source of carbohydrate in a dialyzed horse serum but not in a dialyzed human serum. Subsequently it was shown that the horse serum but not the human serum possesses the ability of hydrolyzing sucrose into glucose and fructose. The implication is clear the results obtained in a particular study may be influenced greatly by the source of the serum used.

I would bring up once again my inability to grow HeLa cells in dialyzed serum diluted in Eagle's basal medium. The ability to propagate HeLa cells in this medium is the whole basis of Dr. Eagle's depletion and repletion experiments. In a more recent publication Dr. Eagle reported that he also was unable to grow HeLa cells in dialyzed serum diluted in Eagle's basal medium as originally described and that inositol is an additional growth factor. He attributed such drastic difference between our results and his results as well as the difference between his earlier and more recent results to the completeness of dialysis. I find it difficult to accept this explanation. By dialyzing 10 cc of serum against 2 changes of 200 cc of saline for only 2 hours the serum no longer would sustain the propagation of the HeLa cells when diluted in Eagle's basal medium as originally described without the inositol. So a dialysis of 2 hours will remove the inositol from the serum. I make this point for only one purpose—to stress an

important weakness of our present cell system. How may one be certain that a cell strain 1 year ago and the same cell strain at present still possesses exactly the same properties? If one does find a distinct change in the nutritional requirements how may one prove conclusively that mutation or adaptation has occurred or that a technical error has been committed? Without a method for the reversible arrest of cell metabolism for an indefinite period of time the systematic study of such a problem is extremely difficult.

Until the time when a synthetic medium capable of supporting growth and a method for the reversible arrest of the metabolism of these human cells become available one should be more cautious in drawing conclusions from experimental data obtained by the methods currently used.

DR. CORIELL: I agree with all the speakers on the panel that when cell lines derived from normal tissue adapt to tissue culture and grow vigorously interesting alterations in morphology, metabolism and reactivity frequently develop. It has been suggested but has not yet been established that these changes are synonymous with becoming malignant.

The criterion of transplantability to cortisone and x-ray treated rats or volunteer cancer patients as described by Dr. Moore should be a reliable test for malignancy but these data also may lead to erroneous conclusions as we have tried to point out by injecting normal monkey kidney cells subcutaneously into treated rats and following the course of the lesion by serial biopsies. In brief the normal cells grow during the first week producing nodules composed almost entirely of epithelial cells which become surrounded by fibroblastic cells and disappear during the third or fourth week.

In our hands the human conjunctiva and kidney lines (Chang) behave like normal monkey kidney whereas HeLa cell nodules persist longer and differ in many microscopic details.

The practical point is that if one examines between 7 and 14 days the nodules from rats injected subcutaneously with normal or malignant cells the microscopic appearance may have similarities which can be misleading. Normal cells have hidden potentialities when placed in the treated animal as Toolan has previously

pointed out. The quantity of cells injected is one important factor but we must realize that in common with all protoplasm even normal cells have a capacity for survival and adaptation to changes in the environment. There is no time here to enumerate the known mechanisms by which this may be accomplished. We have now injected over 1,200 cortisone and x-ray treated rats intraperitoneally with cells from normal monkey kidney 4 malignant cell lines and 3 long-term lines derived from normal human tissue. By this route the distinction between cell lines is very sharp that is malignant cells grow progressively and kill the majority of the rats while the normal cells do not grow at all.

However results with another cell line derived from normal tissue are of considerable interest. When we injected monkeys subcutaneously with the cynomolgus heart cell line (SCH) adapted to tissue culture from normal monkey heart by Silk and Ward a cellular subcutaneous tumor developed which enlarged for 1 to 2 or 3 weeks and then regressed. Great variation was noted in individual monkeys. Pretreatment of the monkeys with cortisone and x-ray did not change the response. Following intraperitoneal injection of these cells no tumors were found in monkeys. In marked contrast this cell line caused massive fatal tumors when injected intraperitoneally into treated rats. SCH cells were even more invasive than HeLa cells and produced nonfatal tumors in untreated rats. These rats were subsequently immune to rechallenge even though pretreated with cortisone and x-ray at the time of rechallenge.

Chromosome analysis of this cell line (SCH) shows that it is heteroploid and quite different from normal monkey kidney. Heteroploidy is not synonymous with malignant change as shown by chromosome counts on Chang con jun via Chang kidney monkey kidney HeLa and SCH cells.

By precipitation and complement fixation tests the SCH cell is closely related to human cell lines (HeLa conjunctiva and kidney (Chang)), and intestine (HeHe) and gives little cross reaction with fresh monkey kidney monkey heart monkey serum or fresh human heart or human serum. This suggests that all long-term established cell lines acquire common antigens not present in the original normal cell

I re-emphasize that temporary survival or growth of transplanted cells in a foreign host or treated animal does not indicate that the cell is malignant. However if the cell grows progressively and kills a cortisone and x-ray treated rat it is certainly malignant for that animal. The true significance of these observations is not clear and much more study is needed. While exploring the meaning of various other methods and techniques of characterizing cell lines I suggest that a cell line may be classified tentatively as malignant if it produces progressive fatal tumors in the treated weanling rat injected by the intraperitoneal route.

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Dr Silk I recall some 6 years ago the  
International Conference in Copenhagen when  
John Enders presented the results of his mag-  
nificent studies with tissue culture which sud-  
denly blossomed to an enormous activity when  
touched with polio virus. Dr Paul predicted  
the end of the monkey era.  
Today's discussions and many of the rest of  
us feel strongly that the end of the monkey era  
will come when the monkey can be replaced  
completely in the preparation of polio virus vac-  
cine and other vaccines as well. The feeling  
was well expressed by Dr Spector when he  
said that the specter of malignancy hovers over  
the question as to whether or not one can use  
an independently propagating cell or self propa-

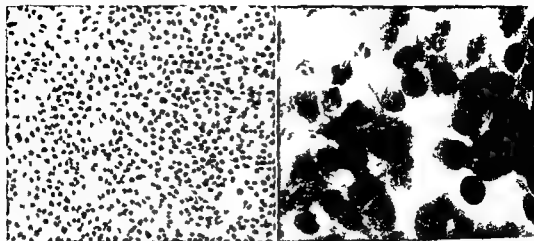


FIG. 179 (Left) Photomicrograph of a culture of the cynomolgus heart cell  
(Right) Higher magnification of the same cell culture

gating cell for the preparation of such material. He urged further that investigations be done rather than resorting to hypotheses and speculation. Dr. Coriell, commenting on Dr. Moore's excellent studies, urged some equivocation as to the definition of malignancy. The question then arises: Is this Conference going to end without some firm and definitive idea that before another Conference or before another year goes out some definite steps will be taken to supplant the monkey kidney in the preparation of polio virus vaccine and thus to eliminate one of the tremendous problems confronting those interested in vaccine technology? Dr. McLinans

presented some excellent studies. Perhaps he and the British workers who introduced the rabbit cell may well have the answer to the question.

In the meantime, quite by chance we encountered the cynomolgus heart cell which Miss Ward observed in the course of cultures being investigated for quite another purpose. When we found some of the properties to which Dr. Coriell has already referred and I shall not repeat, we looked into this question—the question of this cell and its usefulness from the viewpoint of malignancy. Here was a cell that could be tested in the host from which it was

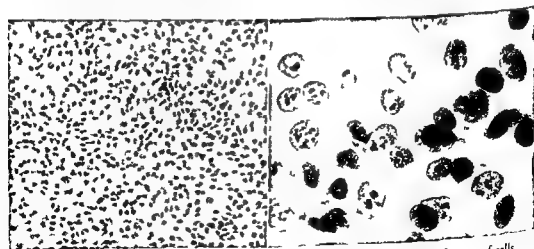


FIG. 180 (Left) Photograph of the 48th subculture (Right) Higher magnification of cells from the 48th transfer



FIG 181 (Top left) Photograph of monkey that was inoculated with 1 000 000 cells subcutaneously (Top right) Stripping of the capsule of the large tumor in the same monkey (Bottom) Cross section of tumor

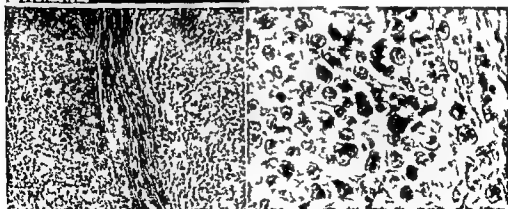
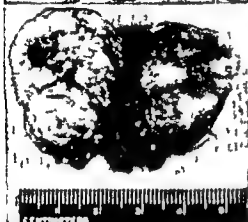


FIG 182 (Left) Photomicrograph of a biopsy of the tumor shown in Figure 181 (Right) Higher magnification of the same tumor

derived a test not so easy to carry out with the HeLa cell or other cells of human origin. I will show the results of our efforts in this direction. Figure 19 left shows a photomicrograph of a culture of the cynomolgus heart cell

You can see the active division that is going on. Mitotic figures are abundant. This is one of the most actively growing cells that those who have compared cell growth have seen. Figure 19 right is a higher magnification of



the same cell culture. This was the 27th transfer. The cell has gone through some 60 or 70 transfers now. Figure 180 left is a photograph of the 48th subculture and you will see the similarity in morphology between this and the first. Figure 180 right is a higher magnification of cells from the 48th transfer.

Figure 181 top left is a photograph of a monkey that was inoculated with 1 000 000 cells subcutaneously some 6 weeks earlier. The lower site was inoculated with  $\frac{1}{4}$  that amount—a million cells, a quarter of a million cells and a quarter of that in the lowest area. This was done in titration. You see the large tumor that developed at the upper site when 1 000 000 cells were inoculated. This was the first time that such an observation had been made. All other times that monkeys had been inoculated we had observed the kinds of tumors that Dr Coriell referred to that lasted a matter of 2 weeks and then disappeared. The large tumor was excised. Figure 181 top right shows the stripping of the capsule; it was fairly free and not adherent. Figure 181 bottom is a cross section of the tumor showing its approximate size. The surgeon who removed it said that to him it would have looked like a benign tumor if he had removed it from a breast. Figure 182 left is a photomicrograph of a biopsy of the tumor; this shows the fibrous part of the fibrous capsule and no evidence of invasion. Figure 182 right is a higher power magnification of this same tumor from which tumor cells were grown.

Figure 183 left is another monkey inoculated at the same time showing smaller tumors. These disappeared ultimately. Other animals in the same group did not develop any tumors. Where 6 sites were inoculated (Fig. 183 right)

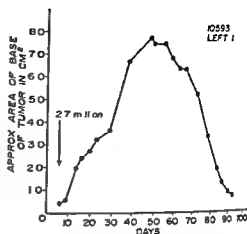


FIG. 184 Growth and regression of subcutaneous tumor in Rhesus monkey induced by cultured monkey heart cell.

and in this instance I believe that these animals received about 2 000 000 to 3 000 000 cells in each site. Tumors of this kind appeared in about 1 out of 20 monkeys so treated. Figure 184 describes the rate of increase and disappearance of tumors when they get to be the size that you just saw. In 40 to 50 days they reached their peak and then disappeared by 90 to 100 days—in about 3 months.

The purpose of this presentation was to show some of the positive evidences that we have of tumor formation following inoculation of monkeys with this particular cell line. We have inoculated hundreds of animals and in no instances have we had either a reappearance of the tumor or anything resembling malignancy. Therefore the question arises as to what criteria are needed to make it possible for us to take the next step in preparing polio virus vaccines from continuous cell lines.



FIG. 183 (Left) Photograph of a monkey showing smaller tumors. (Right) Photograph of another monkey showing tumors which developed at 6 sites of inoculation.

# General Considerations of Viruses

WEDNESDAY AFTERNOON, JULY 10 1957

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Rothamsted Experimental Station  
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## *Discussants*

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DR LARS KJELLÉN

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Houston

# Structural and Functional Parts of Tobacco Mosaic Virus

PROF DR G SCHRAMM

A virus could be defined as a particle containing ribonucleic acid able to invade a host cell and to multiply inside the cell. The question may be raised as to how these biologic functions can be correlated with definite structures of the virus. In bacteriophages and several animal viruses are components which enable these particles to penetrate the cell membrane. They contain substances reacting with the specific receptors in the surface of the cell and enzymes which destroy the receptors. The simple plant viruses apparently do not contain such components. They invade the cell only if the membrane is previously damaged mechanically either artificially by rubbing or naturally by insect vectors. However, we may expect that the external structure of these viruses facilitates the penetration and enhances the stability inside and outside the cell.

The nucleic acid plays the dominant part in the multiplication of viruses as conclusively demonstrated by the work of Hershey and Chase with bacteriophages. It was demonstrated that only the desoxyribonucleic acid (DNA) localized inside the head of the phage is responsible for the multiplication and furnishes the genetic information. The external coat is necessary for adsorption and invasion.

In many other viruses too the inner parts consist of nucleic acid or at least of substances with a high content of nucleic acid whereas the shell is essentially composed of proteins. This was demonstrated for instance for the vaccinia virus for the influenza virus for the virus of fowl plague and for the turnip yellow mosaic virus. The same principle is valid for the structure of tobacco mosaic virus (TMV). Our knowledge about the nucleic acid and protein component of the TMV is particularly developed and will be discussed in this paper.

## STRUCTURE OF TMV PROTEIN

The virus is a rodlike particle with a length of 3000 Å and a molecular weight of about  $40 \times 10^6$ . It contains about 6 per cent ribonucleic acid

(RNA) and 94 per cent protein. The first structural concepts of the protein part were obtained by degradation in weak alkaline solution at pH 10. The kinetics of the degradation is rather complicated and not all the details are clear. The final products are RNA nucleoproteins and a protein component free of RNA called A protein. A protein is homogeneous with respect to sedimentation and electrophoretic mobility. At pH 10 the molecular weight is about 9000. Chemical investigations revealed that A protein is not the smallest subunit of the virus protein. By determination of the terminal groups of the peptide chains it was found that A protein consists of 6 peptide units. Therefore the total protein of the TMV contains about 7300 peptide units of a molecular weight of about 17000. These peptides have the same terminal sequences of amino acids. The sequence of the other amino acids is being investigated in several laboratories and it is possible that all 2300 chains are identical.

It is interesting that A protein can be reaggregated in acid solutions (pH lower than 4) to rodlike particles resembling the original virus. As the electron micrographs show the diameter is the same as that of the virus but the lengths are variable and depend on the pH of the medium. Recently Kramer and Wittmann observed that by reaggregation of the A protein which has a lower anodic electrophoretic mobility than the virus the electrophoretic mobility changes to that of the original virus. The internal structure of the reaggregated A protein was investigated by x-ray methods. The general features of the reaggregated protein and of the virus containing RNA are the same. In both cases the peptide units are arranged in a helix. The reaggregated A protein does not have infectivity due to the lack of RNA.

In the process of reaggregation several distinct steps are observed. The sizes and the shapes of these intermediates and of the final rods can be explained by the properties of the peptide subunits of 17000. This example makes clear that

even complicated biological structures are defined by the properties of very small subunits. Theoretically 2300 subunits can give an enormous number of different recombinations. In fact only one of these is found *in vivo*.

#### ARRANGEMENT OF THE RNA IN TMV

From the alkaline degradation some evidence was obtained about the arrangement of the RNA in the virus. By short treatment at pH 10.3 A protein and free RNA were obtained and also a nucleoprotein with a molecular weight intermediate between TMV and A protein. The nucleic acid content of these particles was higher than in the virus. It was assumed that these particles had lost a part of their protein. This assumption could be confirmed by electron micrographs. In this fraction we observed gaps in the rodlike particles and in these gaps a central strand of RNA was visible. We concluded that the TMV consists of a core of RNA that is embedded in a protein shell like the wick in the wax of the candle. This conclusion was confirmed and elaborated in more details by Hart using a different method of degradation and particularly by the x-ray work of Franklin.

#### FUNCTION OF THE RNA

The virus particles deprived of part of their protein shell had a high infectivity. Therefore at least a part of the protein is apparently dispensable for the process of multiplication. These results lead to the question whether the infectivity of the nucleic acid could be preserved after complete removal of the protein. Because of the instability of the RNA in alkaline solution the preparation of an active RNA by alkaline degradation was not promising. A more suitable method is the extraction of the protein by phenol in a neutral medium. If a solution of TMV is shaken with phenol in the cold the protein is extracted by the phenol phase. After 3 extractions protein is not detectable by chemical means in the RNA fraction. Thus the amount must be less than 14 per cent of the RNA content. Complement fixation showed that the RNA contains less than 0.5 per cent of the native TMV protein. Immediately after such a preparation the RNA is infectious. The average infectivity is about 5 per cent of that of the same weight of the native virus. A comparison of this value with the possible protein content indicates

that the infectivity is not due to contamination with intact TMV or residual parts of the protein shell. Besides the protein determination two other experiments show that the infectivity is not dependent on residual parts of the protein shell. The infectivity is not significantly reduced by repeating the extraction process with phenol and the infectivity of the RNA preparation is not inactivated by TMV antiserum whereas TMV is inactivated under the conditions applied. Other control experiments confirmed that the infectivity is not due to intact virus particles or larger nucleoproteins. The infectivity is destroyed by ribonuclease which does not attack the intact virus. The infective particles have a much lower sedimentation constant than TMV and the stability of the infective particles is much less than that of TMV. Thus we are led to the conclusion that the nucleic acid itself is responsible for the infection. If protein were involved in the process of infection the amount could only be very small and it would not be A protein.

Independently Fraenkel-Conrat found that the RNA is infectious after removal of the protein. By treatment of the virus with dodecyl sulfate he obtained a preparation with a low infectivity. This was destroyed by treatment with ribonuclease and by standing at room temperature. Therefore the infectivity is due to the RNA itself. In the meantime the infectivity of the RNA was confirmed by other laboratories.

The difference between the infectivity of the RNA and the virus is considerable. If we assume that this difference is caused by the function of the protein during the incubation period it should be possible to increase the infectivity of the RNA by recombination with A protein. Recombination of A protein and RNA was tried many years ago in our laboratory, in general the results were negative. By Fraenkel-Conrat and Williams and by Cameron and co-workers an increase of infectivity by recombination was reported. Repeating these experiments we found it difficult to reproduce the reported results. Further investigations are necessary to elucidate the role of the protein part in the process of infection.

Studies have been made to correlate the structure of the RNA with its infectivity. These experiments are difficult because of the instability of the RNA. Frequently a decrease of infectivity was observed after 1 to 2 hours at room

temperature. The reason for this instability is not clear but the manner of preparation of the RNA seems to be of importance. All experiments must be carried out at low temperature and within a short time. The RNA prepared by the phenol method is not homogeneous in the ultracentrifuge. The mean molecular weight can be calculated from  $s_0$  and  $[n]$  as  $1.6 \times 10^6$ . In the sedimentation diagram a sharp boundary can be observed with  $s_0 = 24 S$  ( $c = 0.1\%$ ) followed by a diffuse gradient of material with a lower sedimentation constant. The molecular weight of the material in the sharp boundary can be estimated to be about  $2 \times 10^6$ . The molecular weight of the RNA core can be deduced from analytic data to  $2 \times 10^5$ . The bulk of the infectious material seems to be the undegraded RNA core. By differential ultracentrifugation Gierer demonstrated that in the RNA prepared by the phenol method only the fast moving component with a molecular weight of about  $2 \times 10^5$  is infectious. From his studies some results were obtained about the structure of the RNA in solution. The RNA molecule seems to be a flexible filament with properties intermediate between a rigid rod and a random coil. The high molecular RNA has a lower extinction at 260 millimicrons and a much higher optical rotation than its nucleotides. Probably the nucleotides are arranged in a definite order in the direction of the fiber.

Gierer studied the degradation of the RNA with very dilute ribonuclease in order to decide whether the entire strand of RNA is necessary for the infection or whether some active fragments can be obtained. During incubation with ribonuclease the decrease of viscosity and optical rotation and the increase in absorption was measured. Even a decrease in viscosity of 10 per cent led to a reduction in activity of 65 per cent. Therefore the activity is due to the high molecular material as already shown by differential ultracentrifugation. The high inactivation rate caused by a low degradation rate means that even slightly degraded particles have no more activity. A quantitative calculation reveals that a few breakages, perhaps a single breakage induced by ribonuclease lead to the inactivation of the RNA particle. Thus by splitting normal to the axis no small active subunits can be obtained. That active subunits could be formed by other means cannot be excluded for instance by

separating strands if the RNA were polystriated. This evidence is in line with the result that the TMV rod itself if split into shorter segments loses its infectivity and also with the x-ray investigation which led to a sensitive volume of the order of the total RNA core.

An exact model of the RNA cannot be derived from these studies. The simplest assumption would be a 1 stranded helix in which the nucleotides of adjacent turns are fixed by hydrogen bonds. In the TMV the pitch of the RNA helix is adapted to the helix formed by the peptide units. The length of the virus is defined by the RNA core because by reaggregation of A-protein without RNA definite length is not obtained. The RNA core has a length of 3000 Å and the molecular weight corresponds to about 7000 nucleotides. The peptide unit of TMV contains about 140 amino acids so that the length in the fully extended state would be about 490 Å. If we assume that the RNA is the template on which the peptide is produced directly we have to explain this large ratio. Possibly the RNA has other functions such as the production of enzymes necessary for the protein synthesis. Such functions have been observed with the DNA of bacteriophages.

## CONCLUSION

The investigation of the structure of the TMV virus has also elucidated the function of the structural parts. For multiplication only the RNA is important. It serves as genetic material in the same way as the DNA of the bacteriophages and transforming principles. The properties of the protein produced in the cell are defined by the properties of the RNA. However the protein influences the shape and the stability of the virus. It would be interesting to follow these interactions in more detail and to carry out such experiments with other RNA containing viruses.

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# Studies of Purified Polio Viruses

DR CARLTON E SCHWERDT

I will review some observations made on the purification, identification and characterization of polio virus Types 1, 2 and 3 grown in tissue culture. As Dr Bawden has pointed out these observations are the result of 3 years of investigation carried out at the Virus Laboratory University of California at Berkeley with the cooperation of Dr Frederick L. Schaffer.

## PURIFICATION

The procedure for purifying polio viruses is relatively simple and can be applied directly to large volumes of infectious tissue-culture fluid (TCF) at refrigeration temperatures. The source of virus for these studies was infectious fluid from Marland type cultures of monkey kidney tissue fragments inoculated with Mahoney, MEF 1 or Saukett strains. Assays for following the course of purification were carried out by the plaque technique on monolayer cultures of monkey kidney or human amnion epithelial cells.

Rather than present in detail the basic purification procedure I shall simply enumerate the essential steps as follows: (1) precipitation of the virus from tissue-culture fluid with 15 per cent methanol at pH 4 and elution of the pre-

cipitate with a one fiftieth volume of 0.1 M NaCl solution at pH 9 (2) 2 extractions with n-butanol (3) reprecipitation and elution of virus as in Step 1 but without methanol (4) 1 cycle of high and low speed ultracentrifugation (5) treatment with crystalline ribonuclease (6) a final cycle of high and low speed ultracentrifugation.

Partially purified virus suspensions prepared in this way were 30,000 to 50,000 times more concentrated than the original tissue-culture fluid. The average recovery of infectivity yield of partially purified virus specific infectivity and purification factor for all 3 virus types are summarized in Table 113. I should like to point out in passing particularly to those who are interested in a more potent killed virus vaccine that any one or all of these steps can be applied to the purification and concentration of polio viruses on a commercial scale.

Further purification of the partially purified virus concentrates was effected either by moving boundary electrophoresis or ultracentrifugal sedimentation in a sucrose density gradient. The latter procedure which was more efficient usually fractionated the virus concentrates into clearly discernible components readily harvest-

TABLE 113 AVERAGE RESULTS OF BASIC PURIFICATION OF POLIOMYELITIS VIRUS FROM TCF\*

VIRUS TYPE	% RECOVERY OF INFECTIVITY	YIELD OF PARTIALLY PURIFIED VIRUS (μg / 1 TCF)	SPECIFIC INFECTIVITY†	PURIFICATION FACTOR‡
1 (Mahoney)	40	18	$2.9 \times 10^{11}$	130
2 (MEF 1)	100	24	$1.2 \times 10^{11}$	650
3 (Saukett)	20	11	$1.0 \times 10^{10}$	150

\* These averages represent the arithmetic mean of 11 or 12 purification experiments on each of the 3 concentrates propagated in tissue culture.

† Number of plaque forming units (PFU) / Gm in 1 ml.

‡ Specific infectivity of purified concentrate.

Specific infectivity of TCF

Schwerdt, C. E. Physical and chemical characteristics of purified polioviruses in Cell Culture. In: *Polio Virus and Its Relatives* (Special Publ. No. 5) pp. 15-166. New York: New York Acad. Sci., 1955.





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\* The averages represent the arithmetic mean of 11 or 12 purification experiments on each of the 3 virus strains propagated in tissue culture.

† Number of plaque forming units (PFU)/Gm of concentrate.

‡ Specific infectivity of purified concentrate.

Source: Text of TCF.

Schwerdt, C. E. Physical and chemical characteristics of purified poliovirus in Cellular Biology, Academic Press, New York, 1957, pp. 157-166.

free of one another. These components were designated A, B, C and D in the order of increasing sedimentation rates. The major and fastest sedimenting component contained approximately 95 per cent of the infectivity of the original concentrate. This component freed of sucrose by dialysis was subjected to physical and chemical characterization studies.

### IDENTIFICATION OF THE VIRUS PARTICLE

In order to identify the physical particle with which the property of virus infectivity is associated it is necessary to determine the concentration of the characteristic physical particle present in a virus preparation by some procedure such as quantitative electron microscopy and to correlate this particle concentration with the infectivity titer. The ingenious spray-droplet technique for counting physical particles by electron microscopy developed by Backus and Williams in 1950 has been applied successfully to this problem in the case of the polio virus particle.

This method was first applied to polio virus by Bachrach and Schwerdt who found that Lansing polio virus partially purified from brain and spinal cord ports of infected cotton rats consistently required about 21,000 physical particles per 50 per cent lethal dose in cotton rats. In the more recent work on polio viruses purified from tissue-culture fluid stored for 6 months to 1 year and assayed by the plaque technique on monkey kidney monolayer cultures the ratio of particles to infectious units (plaques) ranged from 1,000 to 2,500. This ratio has been reduced still further as illustrated in Table 114 when in a study with Foch virus was freshly harvested from monolayer cultures of infected cells and assayed for plaques on monolayer cultures of human amnion cells. Under these latter conditions approximately 30 to 35 physical particles of Mahoney virus were found to be equivalent to 1 plaque.

Ideally the ratio of characteristic physical particle to infectious unit should be 1 since the formation of a plaque in tissue culture is probably initiated by 1 infectious particle. This ideal ratio may not have been achieved in any preparation of polio virus as yet because some of the physical particles may have been synthesized in an incomplete form or may have suffered inactivation during manipulation. However, virtually

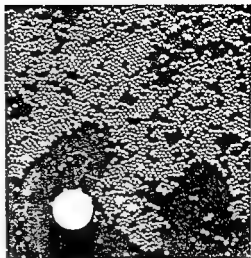


FIG 18> Type 2 (MEF 1) polio virus particles air dried from an ammonium acetate suspension. Particles at the edge of close packed arrays or individual particles are slightly flattened and appear to be larger than those deeper within the arrays. The large white sphere is a polystyrene latex reference particle and is 200 mμ in diameter.

TABLE 114. PHYSICAL PARTICLE TO PLAQUE RATIOS OF POLIO VIRELITIS VIRUS FROM FRESHLY HARVESTED INFECTIOUS TCF

VIRUS TYPE	PHYSICAL PARTICLE/PFU
	35
	38
	46
1 (Mahoney)	34
	28
	41
	22
	32
2 (MEF 1)	60
3 (Saukett)	119

Plaque formation  
 Showed C. E. Th. 1 d h m l h t  
 f p f d pol my l Cell l l l g N l c  
 A l l V sc (Spec 1 P b n 5) pp 157 166 N w  
 Y l N w y l A d s 12)

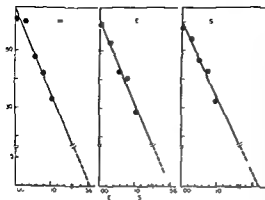


FIG 186 The dry weight density of polio virus particles estimated by plotting the product of sedimentation coefficient and solvent viscosity of highly purified virus suspensions in D<sub>2</sub>O-H<sub>2</sub>O mixtures as a function of solvent density. The straight line was fitted by least squares and extrapolated to zero sedimentation. The dry weight density estimates were 1.57, 1.56 and 1.62 for Mahoney, MEFl and Saukett respectively (Schwerdt, C. E. Cellular Biology, Nucleic Acids and Viruses (Special Pub. No. 5), pp. 157-166, New York: New York Acad. Sc., 1957).

all the characteristic physical particles may have been active after liberation by the infected cell but the existing assay systems may not have been sensitive enough under the conditions employed to detect the infectivity of each particle. At present the latter possibility is favored since continued improvement of conditions of virus preparation and assay has reduced the ratio from 21,000 to 30 particles per infectious unit.

### PHYSICAL PROPERTIES

Both electron microscopic and hydrodynamic methods have been employed to investigate the physical nature of the polio virus particles. Electron microscopy has yielded information about the size and shape of the virus particle. Analytic ultracentrifugation analysis has provided evidence for the homogeneity of the highly purified virus particles as well as data for the estimation of particle density, mass and degree of hydration.

When suspensions of highly purified polio virus particles in a volatile diluent are allowed to dry in air on a collodion film, the particles align themselves in close packed hexagonal

arrays characteristic of uniform spheres. Such geometric formations shadow-cast with uranium are illustrated in Figure 185. If the film is presprayed with a dilute suspension of polystyrene latex reference particles which are uniformly 260 mμ in diameter they may be used for the internal calibration of the fields magnification. Thus the measurement of linear arrays of virus particles along any of the 3 axes of the hexagonal pattern were made and yielded an average particle diameter of about 27 mμ.

Air-dried specimens suffer some distortion due to the surface tension forces exerted at the air-water interface. The effect which is most noticeable on individual particles and particles at the periphery of a 2-dimensional array manifests itself as a slight flattening of the particles. However, this artifact is minimized in the interior of a close packed array. Therefore an estimate of average particle diameter from 6 or more particles within an array should be close to the true diameter. This assumption is supported by data obtained from the measurement of individual virus particles of frozen dried specimens. Such particles which suffered no distortion by surface tension forces since the frozen suspending medium was sublimed away also averaged 27 mμ in diameter.

An analysis of highly purified preparations of Mahoney, MEFl and Saukett polio viruses by analytic ultracentrifugation revealed each to be a homogeneous mono-disperse system with corrected sedimentation coefficients ranging between 157 and 160 Svedberg units. These values were obtained from dilute preparations containing as little as 0.01 to 0.005 per cent protein and therefore were considered sedimentation coefficients at infinite dilution.

The reciprocal partial specific volume or dry weight density of each virus type was determined by analytic ultracentrifugation in various mixtures of D<sub>2</sub>O-H<sub>2</sub>O which were 0.14 molar with respect to NaCl. By plotting the product of sedimentation coefficient and solvent viscosity as a function of solvent density and extrapolating to zero sedimentation, an estimate was made of the solvent density at which no sedimentation would occur. This value which is assumed to be equivalent to the dry weight density of the virus particle ranged between 1.56 and 1.67 for all 3 virus types. Graphic representation of such data is illustrated in Figure 186.

TABLE 115 SOME PHYSICAL PROPERTIES OF POLIOVIRUS VIRUSES

Type	Sedimentation Coefficient	Particle Dry Weight Density	Particle Diameter		Particle Mass	Molecular Weight	Water of Hydration (Gm Water/Cm Dry Weight)
			Anhydrous	Hydrated†			
1 (Mahoney)	160†	1.57	240	273	1.13 × 10 <sup>6</sup>	6.8 × 10 <sup>6</sup>	30
2 (MEF1)	158	1.56	239	270	1.12 × 10 <sup>6</sup>	6.8 × 10 <sup>6</sup>	28
3 (Salkett)	157	1.67	232	272	1.08 × 10 <sup>6</sup>	6.4 × 10 <sup>6</sup>	37

F m h y d e o l y m d i z  
† Γ m e l e c t r o n m i c r o s c o p e d m  
S d b i g t  
S c h d t C. Γ p h y s i c s m o d e l b a s e d o n d f p n d p l m l t m C H J B I g N u c l A d d s  
y k N y k A d s j 57

(50 d l p b n 5) m 15 166 N



FIG 187 Crystals of MEF 1 polio virus particles photographed in visible light. The largest crystals are about  $30\mu$  in length (Schaffer F L and Schwerdt C E. *Proc Nat Acad Sc* 41 1070)

The radius of the anhydrous polio virus particles was estimated by Stokes law making use of the sedimentation coefficient and dry weight density determined experimentally. In this calculation it was necessary to assume that the radius of the hydrated form of the sedimenting particle is equivalent to that of the frozen dried virus particles as measured by electron microscopy.

The electron microscope and hydrodynamic data as well as the virus particle mass molecular weight and approximate degree of hydration estimated therefrom are summarized in Table 115. On the basis of these results we have concluded that there are no measurable differences in these properties among the 3 virus strains studied and therefore among the 3 immunologic types that they represent.

### CRYSTALLIZATION

Further evidence for the high degree of homogeneity of the purified virus preparations is their ability to crystallize under appropriate conditions. The conditions for crystallization were simply sedimentation of the virus particles by ultracentrifugation and storage of the gel like pellet for several days at  $5^\circ\text{C}$  under a small volume of isotonic saline adjusted to pH 5.9.

TABLE 116 CRYSTALLIZATION AND RECRYSTALLIZATION OF MEF 1 POLIOMYELITIS VIRUS

FRACTIONS	RELATIVE SEQUENCE OF FRACTIONATION	SPECIFIC INFECTIVITY
Electrophoretic	1	$7.7 \times 10^6$
Density gradient	2	$3.1 \times 10^6$
Crystalline virus	3	$3.3 \times 10^6$
Mother liquor from 1st crystallization	4	$3.3 \times 10^6$
Recrystallized virus	5	$3.5 \times 10^6$
Mother liquor from recrystallization	6	$3.4 \times 10^6$

Specific infectivity is defined as the number of PFU/ml / unit OD<sub>260</sub> where OD<sub>260</sub> mμ is the optical density at 260 mμ wavelength for a virus particle suspension.

Schaffer F L, C. E. Physical and chemical characteristics of purified poliovirus in Cellular B. 1966. *Adv. Virus Res.* (Society for the Study of Virus Diseases) pp 12-16. New York: New York Acad. Sci. 1967.

The resulting crystals were small tetragonal prisms with pyramidal ends as illustrated in Figure 187.

The largest crystals were approximately  $30\mu$  in length. With careful handling this crystalline virus could be washed free of mother liquor dissolved and finally recrystallized without loss of specific infectivity as compared with that of the purified virus suspension before crystallization. Table 116 summarizes a typical crystallization and recrystallization experiment with MEF 1 virus strain.

The specific infectivity, the infectivity per mass of material, remains constant throughout the crystallization and recrystallization procedure. Dr. Schaffer of the Virus Laboratory of the University of California at Berkeley has kindly permitted me to show a photomicrograph of a crystal of Mahoney polio virus which he has prepared since this manuscript was originally written. These crystals are perfectly formed as shown in Figure 188 and are perhaps 5 to 6 times larger in their linear dimensions than the MEF 1 virus crystals. Dr. Russell Steer of the same laboratory has prepared pre-shadowed replicas of the cut surfaces of such crystals which demonstrate beautifully their internal structure. As you can see in Figure 189 these sections of the crystals reveal uniform particles which are square packed or hexagonally packed.



FIG 188 A crystal of Type 1 polio virus (strain Mahoney). Approximate magnification  $\times 50$  (Steere I L and Schaffer F L. *Biochim et biophys acta* 28:241)



FIG 189 Electron micrograph of replica of fractured polio virus crystal (strain Mahoney). The packing arrangement of the virus particles within the crystal indicates a space lattice of a face-centered cube. Approximate magnification  $\times 25,000$  (Steere I L and Schaffer F L. *Biochim et biophys acta* 28:241)

Some preliminary investigations have been made of the chemical nature of the polio virus particles. Suspensions of MEF 1 virus particles exhibited a typical nucleoprotein spectrum in the ultraviolet range with an absorption maximum of 260  $m\mu$  and a minimum at 241  $m\mu$ . Quantitative orcinol tests revealed a ribonucleic acid content equivalent to 22 to 30 per cent of the total mass of the virus particle while the Cernotti indole color reaction indicated little if any detectable deoxyribonucleic acid. The absence of deoxyribonucleic acid has been supported by the findings of Dulbecco and Vogt who noted that polio viruses are relatively resistant to inactivation by nitrogen mustard which readily inactivates biologically active deoxyribonucleoproteins.

Suspensions of both Mahoney and MEF 1 polio virus particles have been hydrolyzed and quantitatively analyzed for purine and pyrimidine bases by paper chromatography. The results of these analyses are summarized in Table 117. Thymine appears to be totally absent. The molar distributions of the purine bases of both viruses are similar while those of uracil and cytosine are slightly different. Mahoney virus

contains more uracil than cytosine on a molar basis. The converse is found for MEF 1 virus. Confirmation of this small quantitative difference in chemical composition between the 2 viruses requires additional analytic data. If it is real it may be the chemical basis at least

TABLE 117 PURINE AND PYRIMIDINE BASES OF PURIFIED MAHONEY AND MEF 1 POLIOMYELITIS VIRUSES

BASES	MOLAR BASE RATIOS TO TOTAL OF 4	
	MAHONEY	MEF 1†
Guanine	1.07	.98
Adenine	1.22	1.25
Cytosine	.17	.96
Uracil	.99	.81

A r g f s l y e F i p u f i n b y s e l m n  
t u n u s e d t y g r i t t  
t o e l F u l p f i t b y l e c t p h e t c  
l e t t n g t h m n b l m t h o d  
S c h w d e C F P h a l a d h m a l h t t e  
f p f d p l m y l r C e l l u l r B l g y N l i c  
A s i n d v e (S p e c I P I N 5) p p 15 166 N e w  
Y L N w Y k A d S c 1957

indirectly for the immunologic differences among the virus types

The MEF 1 particle appears to contain no carbohydrate by anthrone test other than that accounted for by the nucleic acid content. As yet analyses have not been made for lipids. However the stability of infectivity in the presence of organic solvents and the high dry weight density of the virus particles suggest the absence or low content of this component.

In the light of the limited data accumulated so far on the chemical nature of the purified Mahoney and MEF 1 viruses the particles appear to consist of only protein and ribonucleic acid, the latter component representing 22 to 30 per cent of their total mass. Carbohydrates and deoxyribonucleic acid if present exist in quantities too small to detect by the analytic methods employed.

### ANTIGENIC PROPERTIES

Mayer observed that highly purified preparations of polio virus are type specific by complement fixation test. As antigens these preparations do not cross react with heterotypic sera or with antisera against the constituents of normal noninfected cells of the type used for tissue culture propagation of the virus. When partially purified virus suspensions are fractionated by the method of sedimentation in a sucrose density gradient as described in this paper the fastest sedimenting component, fraction D, is found to contain virtually all the infectivity and most but not all of the type specific complement fixing antigen. The next slower sedimenting component, fraction C, on the other hand contains a measurable amount of antigen but no infectivity. Mayer has also found that acute sera from poliomyelitic patients show a higher complement fixation titer than their respective paired convalescent sera with noninfectious fraction C as antigen, while the converse is true with infectious fraction D.

This suggestion that the fractions C and D are qualitatively different antigens although type specific has been supported by the concurrent findings of Le Bouvier who used our C and D fractions as antigens in the Ouchterlony precipitin test—a technic which involves the double diffusion of antigen and antibody in agar gel. Neither fraction formed a precipitate with heterotypic or antinormal cell sera. However

2 distinct precipitate zones appeared in the agar gel when both antigens were tested simultaneously against homotypic sera.

Fractions C and D differed in physical properties as well as antigenically. Electron microscopy revealed greatly flattened, poorly delineated particles of low electron opacity in fraction C. These particles were slightly larger in diameter than the characteristically uniform spherical virus particles found in fraction D. Ultraviolet spectrophotometry indicated a marked difference in chemical composition between the 2 fractions. The D fraction, as noted earlier, exhibited a typical nucleoprotein spectrum while the spectrum of fraction C indicated the presence of little or no nucleic acid. It must be added that this has been verified recently by chemical analysis.

It is not known as yet whether the slower sedimenting particles of fraction C are noninfectious degradation products of once viable virus particles or are noninfectious particles elaborated directly by the infected cell. More detailed antigenic analyses of both fraction C and D particles in parallel with physical and chemical studies may help to answer this question as well as shed some light on the mechanism of polio virus replication.

### SUMMARY

We visualize the virus particles as being fairly rigid uniform spheres 27 m $\mu$  in diameter with a high dry weight density ranging between 1.56 and 1.62 and containing about 0.3 to 0.4 Gm of water per Gm of dry weight particle in aqueous suspension. The dry weight mass of these particles is about  $11 \times 10^{-17}$  Gm. An estimate of their molecular weight therefore averages 6.7 million. The physical data accumulated so far on all 3 polio virus types does not permit the postulation that their respective characteristic particles exhibit detectable physical differences. However this does not exclude possible differences in surface charge which still must be investigated by electrophoretic methods.

From chemical analyses the physical particles of polio virus Types 1 and 2 appear to consist of only protein and ribonucleic acid, the latter constituent comprising 22 to 30 per cent of the particle mass. There is no evidence as yet for detectable amounts of deoxyribonucleic acid being present.

The purified characteristic virus particles of all 3 types are type specific by complement fixation and by agar gel precipitation tests. However there is isolable from partially purified virus preparations a class of particles which are also type specific by the same serologic tests yet antigenically distinct from the virus particles. These particles which are noninfectious and differ physically and chemically from the virus particles may offer an approach to the study of polio virus replication.

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# *Electron Microscopy of Tobacco Mosaic Virus*

DR ROBLEY C WILLIAMS

## INTRODUCTION

The agent causing mosaic disease of tobacco plants has occupied a unique position with respect to biologic, chemical and physical experimentation ever since its pathogenic characteristics were first described by Mayer<sup>1</sup> and its filtrability by Iwanowski<sup>10</sup> and by Beijerinck.<sup>3</sup> This circumstance is somewhat a matter of historical accident inasmuch as interest in the varied properties of this agent might be expected to result from the unprecedented discovery that it was filtrable, conceivably to the relative exclusion of other agents that might have proved to be as interesting. But as time has gone on it has become apparent that tobacco mosaic virus (TMV) is of uniquely intrinsic importance to those who would like to clarify somewhat the mysteries of the biologic, chemical and physical properties of the viruses. As well as being the first pathogen to be shown to be transmissible after filtration, it was the first to be purified and chemically characterized. TMV was also the first virus to be examined by the methods of physical chemistry, the first to be observed in the electron microscope and the first to be analyzed by means of x-ray crystallography. In recent years it has been subjected to elaborate investigation by x-ray analysis and electron microscopy in an effort to elucidate its structure. The interest in the structure of TMV has been dramatically intensified quite recently by the discoveries that it can be chemically disintegrated into subunits and can be reassembled either as a protein<sup>2</sup> or as a nucleoprotein<sup>9</sup> with a concomitant loss or partial retention of its biologic activity. Since it is now known that its nucleic acid moiety alone is infectious<sup>6,8,11</sup> it is small wonder that the question of its detailed structure assumes considerable importance. I think it is fair to say that to the virologist tobacco mosaic virus stands in the role of the fruit fly to the geneticist, the hydrogen atom to the spectroscopist and the Andromeda nebula to the cosmologist.

## GROSS MORPHOLOGY

The particles of tobacco mosaic virus are apt objects for investigation by the electron microscopist. They are not so large as to be discernibly opaque to the electron beam, yet they are large enough to afford some hope that their structural details can be detected. They are readily purifiable to a high degree, thus relieving the microscopist of his usual and proper doubts concerning the identity of the material being observed. Since the particles of TMV may be taken apart chemically and reassembled, there is the possibility that the electron microscope may be profitably used in following these fascinating reactions. Perhaps most important, there exists a large amount of information about the structure of TMV, drawn from other sources with which the electron microscopist may compare his observations. Although the methods of electron microscopy are not applicable in the small molecular domain where the chemist and the x-ray crystallographer feel comfortable, the direct visualization of somewhat larger structures is useful in affording a check on the implicit expectations arising from experimentation with or indirect observation of the smaller scale units.

I shall refer to tobacco mosaic virus in its extracellular form as simple TMV, although it may be argued that a virus is acting as a virus only when it is in its intracellular state. In the former condition an ordinary suspension of TMV may be shown by the electron microscope to consist of particles that fit this rather rough description: stiff cylindrical rods with a constant diameter of about 180 Å and with variable lengths (Fig. 190). The observation of an apparent nonconsistency of lengths has attracted attention ever since TMV was first photographed.<sup>12</sup> The reason for this attention lay initially in the controversy as to whether TMV (and hence perhaps other viruses) could properly be called molecules. Clearly if there was no uniformity in length the term was not

an apt one while if there was uniformity the chemists would at least have a factual platform from which to argue for the relevance of the term

## DETERMINATIONS OF LENGTH DISTRIBUTION

### INTRACELLULAR VIRUS

The history of experimentation designed to demonstrate microscopically whether or not the particles of TMV are uniform in length is a long and confusing one. As might be expected different people obtained different answers since there was no attempt made to standardize the conditions of preparation. The degree of uniformity of observed length of the particles is dependent on conditions of extraction of the virus from the plant on the mechanical stresses involved in purification on the pH and ionic strength of the suspending medium and on the procedures used in preparing the specimens for microscopy—to name only a few of the pertinent variables. Until 1951 the consensus seemed to be that there was a broad peak in the frequency-distribution curve around a length of 300 m $\mu$  and that the curve was distinctly skewed toward the shorter lengths. A pertinent observation was that the sharpest distributions were obtained subsequent to preparative procedures that were calculated to be the most gentle and followed by suspension in media at pH's near neutrality. One should read Bowden's discussion of the earlier evaluations of length distribution.<sup>1</sup>

Williams and Steere<sup>23</sup> investigated the problem of uniformity of length of the particles of TMV by use of a method particularly designed to correct for any distortion of length-distribution that might be imposed by the forces of drying inevitable in the ordinary procedures of electron microscopy. By using small spray drops for the deposition of the TMV material they were able to obtain micrographs of droplet patterns in each of which typically there would be 3 or 4 particles of uniform length (or twice this length) and only 2 or 3 shorter fragments. After summing the lengths of the fragments and dividing by an integer they found that most of the fragments could be accounted for as uniform length rods that were present initially in the droplets but which were fragmented on drying. From these observations they concluded

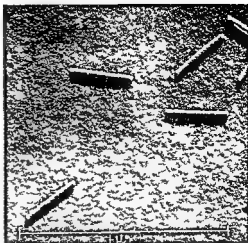


FIG. 190 Electron micrograph of particles of purified tobacco mosaic virus (TMV) showing that the virus particles are generally rodlike of uniform diameter and of reasonably uniform length.

that in their preparations of semipurified material at least 90 per cent of the particles were of uniform length. The preparations were made by homogenization of leaves containing TMV as a fairly young infection (about 7 days) followed only by a treatment of the extracted juice at elevated temperature for a few minutes to coagulate the nonvirus protein.

Apparently then a suspension of TMV can be secured in which almost all the particles are of uniform length. The implication of this finding is obvious unless one is prepared to believe that preparative handling can make uniform particles out of those initially nonuniform. It must be concluded that TMV exists *within the plant cells* in a highly monodisperse condition. Whether or not by various kinds of mishandling one can produce preparations that contain particles of all sorts of lengths has no bearing on this important conclusion; such results merely show that sow's ears can be made out of silk purses.<sup>1</sup>

### INTRACELLULAR VIRUS

More recently it has been possible to show quite directly that at least some of the particles of TMV are of uniform length as they exist within the cell. This demonstration has come

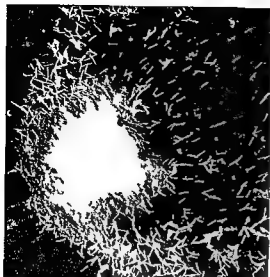


FIG 191 A portion of a partially dissolved inclusion crystal of TMV. The individual virus rods are seen wherever the dissolution has been complete.

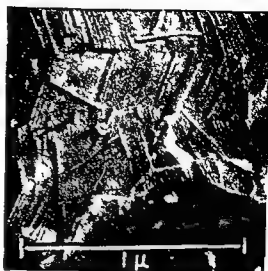


FIG 192 Part of a TMV inclusion crystal that has been frozen, sectioned and its partially sublimed surface replicated. Four tilted layers of closely packed parallel rods are seen.

from an investigation on the identification of the crystalline appearing inclusions found particularly prominently in the hair cells of infected leaves. It has been inferred for some years that these inclusions were composed primarily of virus particles owing to their optical properties when examined in polarized light. By use of these optical methods even the detailed stacking of the presumed virus particles within the crystals has been elaborated.<sup>30</sup> But since the crystals have proved to be refractory to removal without complete disintegration and mixing with plant juice the demonstration that they contain primarily virus particles has proved to be frustratingly inconclusive. However Steere and Williams<sup>7</sup> were able to remove the crystals intact from the hair cells by methods of freeze-drying. Rewetting and dissolving the crystals (Fig. 191) they were able to count the virus particles by electron microscopy and to assay their biologic titer. It was found that substantially all of the dry mass of crystal is virus and that the virus dissolved from the crystal has undiminished infectious titer. Steere<sup>8</sup> has developed a technique by which such a crystal (within its hair cell) can be frozen, transected and the exposed crystal surface replicated for observation by electron microscopy. When such a crystal is transected in a plane parallel with

the direction of length of the TMV particles within a replica shows the particles as they are stacked in situ. They are found in palisades (Fig. 192) with the virus rods in each aligned closely parallel. Every rod in each palisade is found to have the same length—300 m $\mu$ . Whether or not the cell makes any selection of rods for inclusion within the crystals is not known of course so from Steere's beautiful observations alone we cannot conclude that all the TMV particles within the cells are of uniform length. But from this direct type of observation plus the conclusions of Williams and Steere<sup>33</sup> it is probably safe to conclude that in an infected leaf substantially all the virus particles are of uniform length.

It may seem that I have treated the foregoing subject with an undeserved thoroughness. Is it of more than academic interest whether or not the particles of tobacco mosaic virus are of uniform length within plant cells? As we shall see later the question of how long is a particle of TMV is becoming of paramount importance. Since the virus may now be taken apart and reassembled the question arises as to the criteria to employ to know whether or not the reassembly is complete. One criterion is certainly length (or mass since the cross sectional area is de-

monstrably constant) and if it is known that native TMV is uniform in length we are better able to assess physically the excellence of our reassembly operation

### LENGTH OF THE INFECTIVE UNIT

It is apparent though that the nub of the question relating to the uniformity of length of TMV has not yet been discussed. The question of primary relevance is what must be the length of a particle of TMV in order that it can be infective i.e. what is the length of the virus particle? Unfortunately this important question has attracted very little effort directed toward a precise answer. However it is possible at this time to indicate some generalities bearing on the question. It has been known for years<sup>1</sup> that those preparations of TMV that show by electron microscopy (or by any physical method) a preponderance of quite short rods—of length say less than 100  $\mu$ —are relatively noninfectious. In other preparations where the relative numbers of short rods is less the infectivity is greater. However if very long rods are artificially produced in the preparation by dropping the pH the infectivity is somewhat lowered. By infectivity is here meant a number proportional to the number of lesions produced per unit mass of virus. In a general sort of way it may be concluded that infectivity is a maximum for those preparations containing the largest relative abundance of rods of length about 300  $\mu$ . It is to be noted that although short rods in a given preparation can be aggregated in a longer ones by chemical treatment there is no increase in infectivity.

Some work has been done to relate infectivity of TMV with the artificial production of short rods.<sup>21</sup> A preparation initially highly infective and composed in good part of rods 300  $\mu$  in length was exposed to sonic disintegration. It was found that there is a close relationship between loss of infectivity and the relative numbers of broken rods. However the mechanical and chemical effects of sonic disintegration are not thoroughly known and one cannot conclude necessarily that this experimentation proves that the simple breaking of the rods is the sole cause of the lowered infectivity.

Hart<sup>18</sup> has done some work that bears on the question of the minimal length of the infective unit of TMV. He first found that treatment of

TMV with detergent at elevated temperature could be so controlled that the preparations maintained about 25 per cent of their initial activity. But after exposure of the treated preparation to ribonuclease the infectivity was found to drop further by at least a factor of 10 while the controls dropped by only a factor of 2. Since Hart previously had found by electron microscopy that more prolonged treatment with hot detergent laid bare a portion of the RNA of the virus<sup>15</sup> the most likely interpretation of the biologic effects of brief treatment followed by ribonuclease is that the detergent exposed a small portion of the RNA and that the enzyme digested this portion i.e. that the structural integrity of the entire PNA portion of the virus is necessary for infectivity. This conclusion is consistent with the notion that nearly if not exactly a full length rod is the viable unit and that this length is probably the 300  $\mu$  one generally found in abundance in gently prepared extracts. It is my personal conviction that rods of native TMV are potentially infective only if they are near to this length but I can offer no quantitative limits on the degree of nearness required.

### DETAILED STRUCTURAL FEATURES

The general morphology of the rods of TMV as observed by electron microscopy is easily described. They are seen to have a most frequent length of  $2980 \pm 10$  Å<sup>22</sup> a diameter of about 180 Å when observed as single particles and a center-to-center separation of 150 Å when measured in packed parallel arrays. (The inconsistency implicit in the latter two statements will be discussed subsequently.) The general shape is that of a stiff rod with blunt ends. On a finer scale of size which might still be amenable to electron microscopic examination there are at least 3 questions to ask: what is the cross sectional shape of the virus rod; is there any periodic structure along the surface of the rod; is there any internal structure? As we shall see the existing answers to these questions are not unequivocal nor are they wholly consistent with the conclusions drawn from x-ray analysis of TMV structure.

### SURFACE STRUCTURE

It might be expected that high resolution electron microscopy of the intact virus particles as

they are dried on the specimen support from purified suspensions would delineate their cross sectional form and surface structure. But in order to achieve sufficient electron contrast for an object as small as this to be seen clearly it is necessary to shadow the specimen with some heavy metal such as uranium. When this is done certain anomalous features of the electron image appear the consequences of which are certainly frustrating and the causes of which are not wholly certain.<sup>31</sup> It is my guess that inasmuch as the shadowing operation is carried out in a vacuum maintained by pumps containing oil the specimen surface becomes coated with tiny ( $\sim 30$  Å) droplets of oil prior to the deposition of the shadowing metal. As a consequence at this level of size or smaller all surfaces look alike in exhibiting a pebbly structure (the metal shadowed oil droplets). Further there appears to be some migration of the metal-coated oil with the result that light and shade in the shadowed specimen cannot be interpreted to be correlated closely with the angle between the surface shadowed and the direction to the source of evaporating metal. Whatever the causes may be it is by now well known that electron micrographs of the surfaces of the particles of TMV

do not show any periodicities of structure. do the particles exhibit any unique sectional shape (such as a circle a square a hexagon) when photographed in the ordinary way (Fig. 193).

In an attempt to obviate one of the difficulties mentioned Williams<sup>31</sup> broke particles of TMV into very short lengths ( $\sim 60$  Å) by sonication and photographed these segments end-on. It was observed that a large fraction of the short pieces of TMV were hexagonal in contour while the others were mostly distorted disks. Williams assumed that the artifacts introduced by the vagaries of shadowing would be more likely to produce distorted shapes out of initially perfect hexagons than the other way round and concluded that it was likely that the cross sectional shape of the TMV rod was hexagonal. However more recent results obtained from x-ray analysis cast considerable doubt on this conclusion.

Any discussion of the structure of TMV revealed by electron microscopy must include the correlative findings from the x-ray laboratories. The first x-ray examination made of regularly oriented gels of TMV<sup>4</sup> showed the particles to have a center-to-center spacing

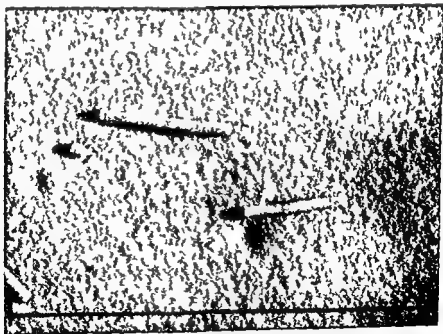


FIG. 193. A high resolution electron micrograph of TMV. No evidence of periodic surface structure is found.

150 Å in the dried gel state and also that there was some structural repeating unit of 68 Å length along the particle axis. In more recent years the x-ray structure of TMV has been examined quite intensively.<sup>10,11,12</sup> The most striking conclusion from this new work is that the virus rod as a whole is a helix with a pitch of 23 Å. Structural subunits are found roughly corresponding in size to the known chemical subunits<sup>7,13,14</sup> and there are believed to be  $16\frac{2}{3}$  of these per turn of the helix. There is also evidence for deep grooving along the helix; the external appearance of the rod would be like that of a rope wound helically around a small cylinder (Fig. 194). It is evident from what has been discussed that the electron microscope has failed to disclose the two primary aspects of surface appearance predicted from the x-ray analysis: a generally circular cross-sectional shape of the TMV rod and a periodic 23 Å cross-striation due to the grooves. The lack of confirmation of the grooving may well be due to the difficulties associated with shadowing as discussed above, but as yet there seems to be no way to reconcile the helical structure (with  $16\frac{2}{3}$  subunits per turn) with the observed hexagonal shape of thin segments of the virus rod.

#### INTERNAL STRUCTURE

When we consider the radial distribution of material within the virus rods there is much better agreement between the x-ray and the electron microscopic conclusions. The main features of the x-ray determination of radial density distribution are: (1) from the axis to a radius of 20 Å the rod is hollow (?); at 40 Å radius there is a region of high x-ray scattering, believed to be associated with the phosphorus of the viral RNA; and (3) the x-ray scattering material extends outward to a radius of 95 Å.<sup>10</sup>

The existence of the predicted hollow cylinder along the axis of the TMV rod has been elegantly confirmed by electron microscopy.<sup>15</sup> Huxley found that it was possible to delineate the inner wall of the cylinder by allowing some electron-opaque material such as a phosphotungstic acid solution to enter the cylinder and then to dry leaving a coating of dense metal along its inner wall. Micrographs of unshaded TMV so treated show 2 parallel lines some 50 Å apart—exactly the appearance that

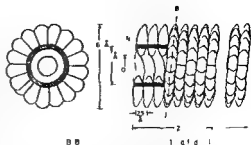


FIG. 194. A representation of the helical structure of TMV derived mainly from x-ray analysis. The cross-hatched region shows the nucleic acid at its proper radial position but its disposition along the helix is shown only schematically.

one would expect if a hollow surface-stained cylinder were present.

The localization of the RNA within the particle of TMV has been sought for some time by electron microscopists. It was not expected that it would be found easily since only 6 per cent of the mass of the particle is RNA and since there is strong evidence that the intact virus rod cannot be chemically stained by any known method. But during some observations by Rice *et al.* of the effects of freeze-drying on the particles of TMV, it was noted that an occasional rod was segmented with the segments lying in a straight line and separated by appreciable intervals. Between segments could be seen a fine fiber along the axis of the rod. It was speculated that this fiber ( $\sim 40$  Å in diameter) was the RNA of the virus rod—a speculation for which there was no good reason at the time but which later has been shown to be a valid one. Schramm and his colleagues<sup>4</sup> have reported that in preparations of TMV partially degraded at high pH there frequently was seen fibrillar material of a diameter such as to suggest that the fibers were RNA. Although no chemical test was made to ascertain the validity of this suggestion, it was a plausible one since the fibers appeared to be coaxial with the undegraded remnants of the virus rods and since the fibers must be either RNA or else a curiously fibrillar form of partially degraded protein. It remained for Hart<sup>16</sup> to demonstrate that the coaxially localized fibers were viral RNA. He treated the virus to a detergent solution at ele

vated temperature quickly chilled the material and observed in the electron micrographs that each partially degraded virus rod retained an axially localized fiber at one or both ends (Fig 195). More significantly, an implicit chemical identification of the fibers with RNA was accomplished by treating the partially degraded material with ribonuclease and noting that the fibers then disappeared whereas treatment with other enzymes did not effect their disappearance.

There is even further electron microscopic evidence of a somewhat indirect nature for the localization of the RNA within the particles of TMV. Schramm<sup>23</sup> has shown that TMV can be partially degraded with alkali to produce particles of weight about 100 000 (the A protein) and free of RNA. When such particles are examined in the electron microscope they appear to be disklike but in contrast to the disks or segments produced by sonic disintegration of the native virus they have a central hole about 60 Å in diameter (Fig 196). Similar appearing disks have been photographed by Takahashi and Ishii<sup>6</sup> in preparations of the so-called X protein obtained from the juice of infected plants from which the TMV had been removed by sedimentation. The partially polymerized X protein like the A protein is devoid of RNA.

It may appear surprising that the determinations of radial density distribution show that x ray scattering material extends to a radius of about 95 Å (diameter = 190 Å) whereas both x ray and electron microscope examination of the virus in packed arrays shows the center-to-center separation of the particles (and hence their diameters) to be precisely 150 Å. An ingenious way out of this apparent anomaly has been proposed<sup>10</sup> based on the evidence that the rods are deeply grooved. The outer radial limit of x ray scattering material would correspond to the ridges of the grooved contour but in packed arrays it would be expected that the rods would fit together ridge to hollow like two machine screws of the same pitch brought together in close parallel contact. In this latter case the diameter of a TMV rod would be some sort of mean between the minimal value determined by the depth of the hollows and the maximal value determined by the height of the ridges. As a matter of fact there is some indirect electron microscopic evidence for the existence of grooving<sup>3</sup> despite the lack of direct evidence of surface periodicities discussed previously. It is found that individual isolated particles of TMV are not 150 Å in diameter but are about 180 Å a value that can be thought of as corresponding to the maximal diameter



FIG 195 A preparation of TMV that has been heated briefly in a weak detergent solution. Part of each rod has been dissolved disclosing the RNA as coaxially localized fibers.

determined from curves of radial density distribution

### SUMMARY

From the observation of the structure of TMV by methods of chemistry x ray analysis and electron microscopy there emerges the following picture TMV is a nucleoprotein containing some 18 amino acids in its protein portion with its 6 per cent of nucleic acid of the ribose type The protein may be disassembled to an apparently homogeneous subunit with a molecular weight of about 17 000 presumably these are the building blocks Evidently the chemical subunits also have a structural significance since their presence is shown in the x ray patterns where they appear as the fundamental unit in the helical structure The protein portion of the virus is assembled in the form of a helix with 49 subunits in 3 turns and the pitch of the helix is 23 Å The exterior of the helix is believed to be deeply grooved The length of the most commonly observed virus particle is 2980 Å while the diameter (discussed above) is either about 180 Å or 150 Å depending on whether the isolated or the packed virus is considered The central portion of the virus rod is a hollow cylinder 20 Å in radius Immediately outside this hollow tube is a layer of protein in a structural array not known but at a radius of 40 Å there is believed to be resident the phosphorus atoms of the RNA The long-chain PNA molecules are certainly known to be localized coaxially with the rod but the detailed configuration assumed by the RNA is not known There is only protein between the radial locus of the RNA and the outer edge of the rod The cross sectional shape is uncertain but is probably generally circular although not smoothly so When we reflect that almost nothing was known about the structure of TMV 10 years ago except for the observations that it was a nucleoprotein containing 6 per cent RNA and that it was rod like in shape with a diameter of 150 Å we can appreciate that within the last decade this small corner of the virus world has received considerable illumination

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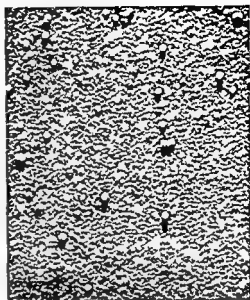


FIG 196 Small particles of protein that come from detergent treated TMV after precipitation of the RNA Most of these are very short cross sections of the initial virus rods and contain central holes These holes are believed to reflect the absence of the virus PNA

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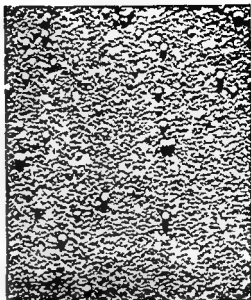


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# *Electron Microscopic Studies of Animal Viruses in Thin Sections*

DR. COUNCILMAN MORGAN

Initial attempts to visualize viruses in cells employed whole mounts of tissue cultures. Although intracytoplasmic particles were encountered they could not be identified as such with any degree of certainty. The introduction of methacrylate for embedding specimens enabled relatively thin sections to be cut on modified standard microtomes. Tobacco mosaic virus was the first to be observed within sectioned cells and shortly thereafter fowl pox, influenza, adenovirus and molluscum contagiosum were also seen. Because the sections were too thick to permit adequate resolution the embedding plastic had to be removed with solvents. The resulting distortion obliterated most of the fine structure

of the cell as well as internal components of the viruses.

Pecognition of buffered osmium tetroxide as a suitable fixative (with subsequent modification to render it isotonic) and the development of special microtome permitted detailed electron microscopic study of viruses within cells. The first viruses to be examined were these new techniques were adenovirus, ectromelia and molluscum contagiosum.

Rather than re-examine the numerous excellent papers published during the past few years 5 viruses studied in our laboratory will be discussed in some detail. The following remarks concern work in progress and are intended solely

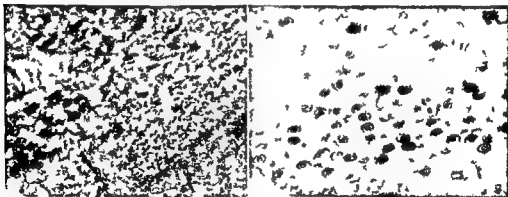


FIG. 19 (Left)  $\times 74,000$  (Right)  $\times 39,000$



FIG. 198 (Left)  $\times 83,000$  (Right)  $\times 48,000$

- 15 Hart R G Proc Nat Acad Sc 41 261 1955
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# *Electron Microscopic Studies of Animal Viruses in Thin Sections*

DR COUNCILMAN MORGAN

Initial attempts to visualize viruses in cells employed whole amounts of tissue cultures. Although intracytoplasmic particles were encountered they could not be identified as virus with any degree of certainty. The introduction of methacrylate for embedding specimens enabled relatively thin sections to be cut on modified standard microtomes. Tobacco mosaic virus was the first to be observed within sectioned cells and shortly thereafter fowl pox, influenza, vaccinia and molluscum contagiosum were also seen. Because the sections were too thick to permit adequate resolution, the embedding plastic had to be removed with solvents. The resulting distortion obliterated most of the fine structure of the cell as well as internal components of the viruses.

Recognition of buffered osmium tetroxide as a suitable fixative (with subsequent modification to render it isotonic) and the development of special microtomes permitted detailed electron microscopic study of viruses within cells. The first viruses to be examined with these new techniques were vaccinia, ectromelia and molluscum contagiosum.

It is rather than review the numerous excellent papers published during the past few years 5 viruses studied in our laboratory will be discussed in some detail. The following remarks concern work in progress and are intended solely

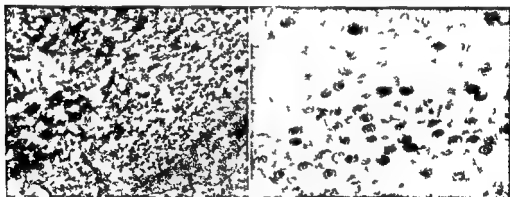


FIG 197 (Left)  $\times 24,000$  (Right)  $\times 39,000$



FIG 198 (Left)  $\times 83,000$  (Right)  $\times 48,000$

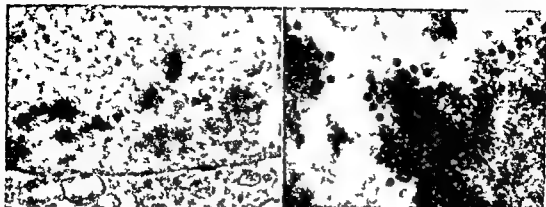


FIG 199 (*Left*)  $\times 9\,000$  (*Right*)  $\times 38\,000$

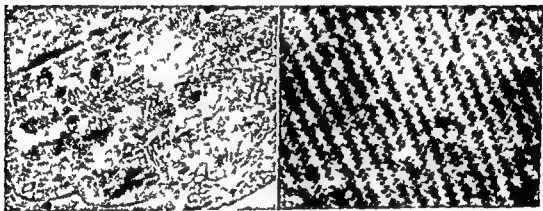


FIG 200 (*Left*)  $\times 10\,000$  (*Right*)  $\times 25\,000$

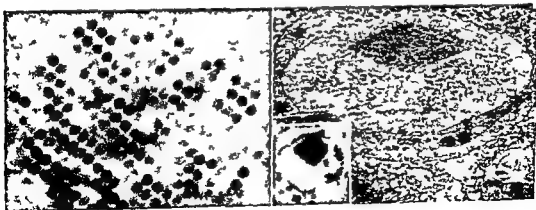


FIG 201 (*Left*)  $\times 50\,000$  (*Right*)  $\times 3\,000$

to indicate the type of information that can be obtained from these sections. I do not presume to suggest that these studies are definitive.

# HEPPES SIMTLEY VIRUS IN THE CHICKEN CHOI IO ALL INTOIC MEMBRANE

Marginal on of reticular material is the initial change observed with infected cells. The nucleus illustrated in Figure 197 left possesses a distinct nucleus limiting membrane (M). In the right half of the field small particles averaging 400 Å in diameter and exhibiting centers of low density partially replace the magnated reticulum. Scattered through the nuclear matrix are larger viral particles composed of an inner body and a dense limiting membrane. Within the cytoplasm a particle (P) has a double peripheral membrane. Figure 197 right shows an intranuclear aggregate of particles many of which have incomplete membranes. Virus with double limiting membranes (Fig 198 left) and averaging 1,200 to 1,300 Å was encountered only in the cytoplasm and in the extracellular space. Such observations suggest that from magnated reticulum within the nucleus the virus differentiates as a small (400 Å) particle that increases in size and is enclosed by a single membrane (Fig 198 right) that on release from the nucleus it acquires a second membrane and that this form represents the completed infectious unit which is encountered in the extracellular space.

## ADENOVIRUSES IN HELA CELLS

Clusters of viral particles are frequently observed adjacent to intranuclear aggregates of dense reticular material (Fig. 199 left) (The nuclear membrane is the lower border of the field). This spatial orientation of the virus together with the poorly delineated particles visible in the reticulum when examined at higher magnification (Fig. 199 right) suggests that the virus is different from the dense

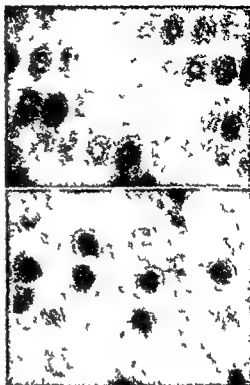


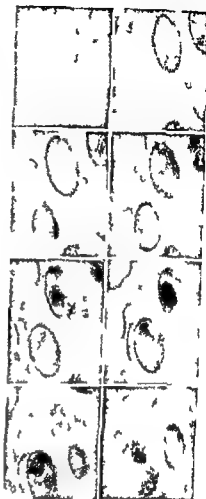
FIG. 07 (Top)  $\times 130,000$   
(Bottom)  $\times 135,000$

multiple intergrowth crystals (Fig. 200) composed of small particles arranged in a cubic body-centered lattice. The orientation of the lattice with respect to the plane of section will largely determine the pattern observed in the micrograph (Figure 200). Right for example illustrates part of a single crystal. The narrow zones of clearly defined particles indicate regions parallel to the plane of section where the zones of low density and poor development of regions cut eccentrically. The particle average 100 Å in diameter with a center-to-center spacing of approximately 650 Å. At higher magnification, two morphologic types may be distinguished (Fig. 201 left): dense particles with little internal structure and others less dense with a central body. Such structural differences may reflect differences in function and raise the question whether only one type represents the infectious unit. If this were the case, it could explain the





FIG. 203 (Left)  $\times 170,000$   
(Right)  $\times 57,000$



discrepancy between particle counts and infectivity noted by Hilleman. In exceptionally thin section (Fig. 207 top) the central body is revealed as an area of low density enclosed by a thin membrane. On the other hand after formalin fixation a relatively large and uniformly dense central body is observed (Fig. 207 bottom).

Adenoviruses undergo no change in structure after release from the nucleus suggesting that further development does not occur in the cytoplasm. Chemical analyses of these viruses are not available but the crystals of Types 3, 4 and 7 are Feulgen positive. Figure 201 right illustrates the nucleus of a cell containing a rhomboidal crystal composed of viral particles. The inset at the lower left shows the same nucleus in a contiguous thick section photographed by light microscopy after Feulgen staining. The crystal is strongly Feulgen positive indicating that the virus contains deoxyribonucleic acid.

#### FOWL POX AND VACCINIA VIRUSES IN THE CHORIOALLANTOIC MEMBRANE

Unlike herpes simplex and the adenoviruses, vaccinia and fowl pox viruses appear to develop entirely within the cytoplasm. Figure 203 left shows 3 particles of vaccinia. One exhibiting a single limiting membrane contains a dense reticular body separated from the matrix by an irregular zone of lesser density. Serial sections reveal that the other particles undoubtedly have

been cut at a level removed from the dense body. The 8 consecutive sections illustrated in Figure 203 right demonstrate (1) that the dense internal body will be transected only when it lies at the appropriate level, (2) that a particle cut eccentrically exhibits a poorly defined limiting membrane due to its oblique position with the section, and (3) that once the virus was probably spherical before distortion by the microtome knife and measures 2000 to 2400 Å in diameter, the average section thickness approximately 300 Å. Fowl pox virus is shown in Figure 204 left. Although its general structure is similar to vaccinia at this stage of development a double limiting membrane is visible. At the left margin 2 incomplete viral membranes are contiguous to an aggregate of granular material. A similar though somewhat larger aggregate is

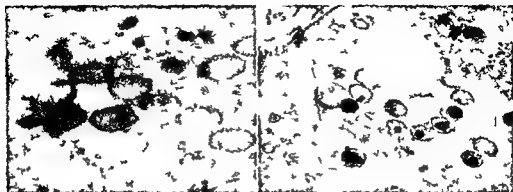


FIG 704 (Left)  $\times 39,000$  (Right)  $\times 74,000$

illustrated by Figure 704 (right). The membranes are incomplete on the side adjacent to the granules and nearby characteristic viral particles are visible. Serial sections through such structures reveal that the incomplete membranes are not artifacts produced by the microtome knife. Moreover, similar structures have been encountered in a different host. Figure 705 (left) shows a section through rabbit skin infected with vaccinia virus and again granules partially surrounded by incomplete membranes are evident at the upper right corner. Such observations suggest that these intracytoplasmic structures are of viral differentiation.

In this connection Bernhard *et al.* observed densities in the study of the Shope fibroma virus. Since a certain fowl pox ectromel and the Shope fibroma virus possess similar internal components, would hardly be surprising in

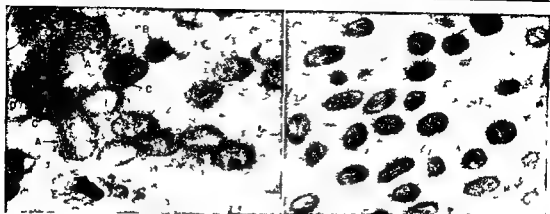
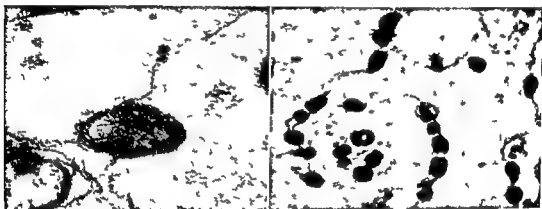
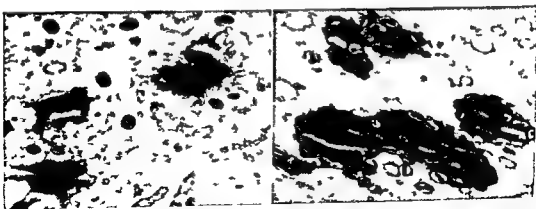
find that they become differentiated in the same manner.

Forms considered to be intermediary in the subsequent cycle of development are encountered infrequently, implying that the changes occur rather quickly. Figure 706 (left) illustrates presumed developmental forms (marked by A) of fowl pox virus. Study of similar fields indicates the following sequence: Dense reticular material (B) incorporated during differentiation of the viral membranes comprises the dense internal body (C). This body enlarges as limiting membrane forms (D)\* and the remaining reticular material disappears (E). Serial sections of a particle similar to F (Figure 707 right) reveal a central body in the form of a condensed disk enclosed by a double membrane and separated

See also in part by Figure 708.



FIG 705 (Left)  $\times 33,000$  (Right)  $\times 80,000$

FIG 206 (Left)  $\times 30\,000$  (Right)  $\times 37\,000$ FIG 207 (Left)  $\times 85\,000$  (Right)  $\times 74\,000$ FIG 208 (Left)  $\times 18\,000$  (Right)  $\times 16\,000$

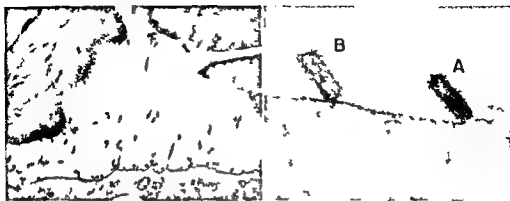


FIG 209 (Left)  $\times 10,000$  (Right)  $\times 97,000$

from the peripheral viral membrane by a zone of low density

Vaccinia exhibits slightly different structure at this stage of development a fact not previously recognized. As shown by Figure 206 right dense material occupies the zone between the internal body and the peripheral membrane. The shape of these particles is best explained by assuming that they are oblate spheroids oriented at random within the section.

Vaccinia and fowl pox near the cell surface possess a double limiting membrane. In Figure 207 left cytoplasm occupies the right portion of the field the extracellular space is on the left. A vaccinia particle appears to be in process of migration through the host-cell wall. In Figure 207 right 5 vaccinia particles are visible within the cytoplasm whereas the remainder occupy the intracellular space between contiguous cells.† The presumption that this form represents the completed infectious unit is supported by the observation of particles having similar structure in sections of pellets prepared by ultracentrifugation of infective chorioallantoic fluid.

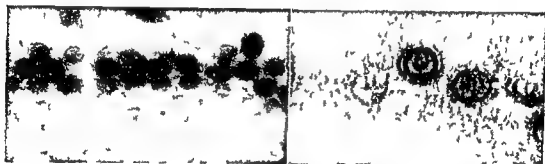
Of interest are the aggregates of fowl pox virus at the periphery of dense intracytoplasmic bodies first observed by Eassey and Flewett and assumed by them to represent inclusion bodies. We did not see similar structures in our initial studies. However osmophilic material is en-

countered repeatedly in the cytoplasm of a variety of cells and generally accumulates during cellular necrosis. Figure 208 left for example shows 3 irregularly shaped dense bodies near particles of vaccinia. Flewett's strain of virus (which he was kind enough to send us) appears to destroy the cells relatively slowly therefore large amounts of the dense intracytoplasmic material accumulate frequently incorporating those viral particles which happen to lie in proximity (Figure 208 right). Whether such structures constitute inclusion bodies remains to be determined. In any case they do not appear to play an essential role in viral development.

### INFLUENZA VIRUS ON THE CHORIO ALLANTOIC MEMBRANE

The spherical and filamentous forms of influenza virus develop at the cell surface. In Figure 209 left an endodermal cell occupies the lower border. Numerous viral particles are in process of release from the free surface. Others are attached to 3 red blood cells occupying the upper portion of the field. When viewed at sufficient magnification the interior of some filaments appears to be continuous with the host cell cytoplasm (Figure 209 right filament A) while the point of attachment of others is revealed as a narrow stalk (Figure 209 right filament B). It appears likely that the filaments form by a process of extrusion from the cell wall that subsequently the membrane of the filament encloses the end proximal to the cell and that the intact filament is then released.

† The possibility that the latter described material is composed of debris from the host cell is not ruled out by the fact that it is not seen in sections of infected cells prepared by the method of Eassey and Flewett.

FIG 210 (Left)  $\times 28\,000$  (Right)  $\times 43\,000$ FIG 211 (Left)  $\times 83\,000$  (Right)  $\times 90\,000$ 

into the chorioallantoic fluid. The filaments vary in length as shown in Figure 210 left. Here short filamentous forms line the surface of the host cell, whereas a bundle of long filaments extends upward near the right border. Such filaments although not exhibiting internal structure possess a dense limiting membrane and a peripheral coat of low density.

Frequently the spheres are confined to a portion of the cell surface which is devoid of filamentous forms. In Figure 210 right, for example, the spheres on the left are separated from the filaments on the right. At higher magnification (Fig 211 left) the spheres although also possessing a limiting membrane and an ill defined peripheral coat can be seen to contain one or several internal bodies. The indistinct particles just beneath the cell wall which traverses the field horizontally exhibit incomplete membranes on that aspect nearest the interior

of the cell and are believed to represent spheres in process of formation.

Figure 211 right illustrates a sphere which is probably central to the plane of a comparatively thick section. Its structure is clearly visible.

These and similar observations suggest the following conclusions:

- 1 The spheres develop in a manner which is different and distinct from that of the filaments.
- 2 The spheres possess one or more internal bodies, whereas the filaments lack visible internal structure.
- 3 The limiting membrane and the external coat of both are similar.

If one then makes the assumption that both forms of the virus can act on the surface of red blood cells to cause agglutination but that only the spheres with internal components are capable of initiating infection, two recent puzzling observations are clarified. Donald and Isaacs em

playing ultrasonic vibration succeeded in fragmenting the filaments. They noted a rise in hemagglutinating activity but no appreciable change in infectivity. Burnet observed no significant loss of infectivity after destruction of the filaments. Although the concept that the sphere constitutes the infectious unit of influenza virus is consistent with the available experimental data, extensive investigation will be necessary to substantiate such a hypothesis.

#### WESTERN EQUINE ENCEPHALOMYELITIS VIRUS IN HUMAN AMNION CELLS\*

Small primary bodies (c. 200 Å in diameter) appear to differ from within the cytoplasm of the host cell. After release the virus exhibits a dense central body averaging 100 Å in diameter, a sharply defined limiting membrane of low density approximately 350 Å in diameter and a diffuse peripheral coat (Fig. 217).

#### CONCLUSION

Examination of morphology provides some insight into the manner of viral differentiation, but structure unless correlated with function remains static. If ultimately the mechanisms whereby animal viruses develop are to be understood, techniques similar to those employed so successfully for the study of tobacco mosaic virus must be adapted and extended. The chemical nature, immunologic properties and effect on host cells might thus be determined for different structural components.

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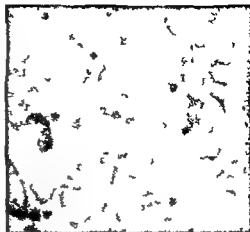


Fig. 217  $\times 89,000$

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## DISCUSSION

Dr FRAENKEL CONRAT As Dr Bawden has said there is remarkably good agreement in the field of virology as far as the nucleic acid of TMV is concerned. I have something to add to that which will prove that the agreement is slightly better than we thought 6 months ago. Dr Schramm has described his nucleic acid as corresponding to the complete complement of nucleic acid in one particle. The molecular weight of about 2 000 000 corresponds to the weight of the nucleic acid of one particle whereas preparations that have been made by our detergent technique have been found to resemble more the earlier described preparations of Cohen and Stanley of a molecular weight of about 200 000—one tenth to one twelfth of the whole complement.

Recent studies have shown clearly that these two preparations do not differ but that what differs is the way in which you look at them. If one ultracentrifuges nucleic acid in 0.07 M phosphate buffer as Schramm has done and if it is good nucleic acid it will act as if it were big. If one ultracentrifuges it in very dilute phosphate or water it acts as if it were small. And so he was right and we were right and we still do not know who is right because the question is now: Is it really big? It refuses to sediment normally only in the absence of ions through charge effects which are poorly understood by the physical chemists. Can such enormous differences be due to such charge effects or is it really small but aggregates through the presence of the salt? We still do not know the answer to that question. We know that we are both looking at the same animal but we do not know quite how big the animal is. We still favor the idea that it is small from independent evidence which I do not want to go into now, part of which was alluded to by Dr Williams in that we have made nucleic acid from sonically inactivated virus. Sonic treatment breaks the rods; therefore you should be unable to get complete nucleic acid complement out of any rods. They are broken yet we get more activity out of such preparations than one would expect. So we still think that the stuff is really small and

aggregates in salt but we have little evidence as has the other side about the real nature of the monomer. That it may aggregate to bigger things is certain.

The study of molecular size in salt has led to other observations which are interesting in that if you keep the nucleic acid at a high ionic strength for any length of time it then loses activity. That has been found both by Dr Schramm and by us and we have now found that as it loses activity it also loses its molecular size, shape, state of aggregation whatever you want to call it. It gets again smaller. Apparently there is a degradation occurring due to the higher salt concentration and requiring time and temperature. This is an effect different from the instantaneous aggregation that I first described.

All salts in tenth molar concentration will cause this degradation with the exception of one that we have found so far and that is pyrophosphate. It is very interesting because 6 months ago we described how the reconstitution of active virus from nucleic acid and protein occurs remarkably well in pyrophosphate as a buffer. We described 6 months ago that you can get 30 to 50 per cent of the activity of the nucleic acid back by reconstitution the same amount of nucleic acid originally in the naked virus. Dr Schramm said that if this nucleic acid is really only less active when isolated because it is naked then putting it back in its coat should make it again as active and we do think that we are achieving that to the extent of 30 to 50 per cent of the ideal. That is possible only in the presence of pyrophosphate and it is interesting that pyrophosphate is later found to be the salt that will not permit the nucleic acid to become degraded. So it seems that the reconstituting reaction is one in which you are fighting a losing battle against the rate of decomposition of the nucleic acid. You are trying to make rods of protein yet you lose activity of the nucleic acid through its sensitivity to salt and by choosing a salt which is less harmful to the nucleic acid you get highest reconstituted activities.



One additional minor point is that recently we have found that the pyrophosphate actually is bound to the nucleic acid when you use radio active pyrophosphate and radioactive phosphate and then dialyze nucleic acid after it has been treated with either of those salts then the pyrophosphate stays with the nucleic acid remarkably more efficiently than the phosphate and it does not dialyze away to the extent of one pyrophosphate residue per nuclear type being held. Therefore there is a definite interaction of pyrophosphate with nucleic acid which probably accounts for these observations.

DR. L. JELLEN: As we reported in 1955 electron microscopy of thin sections of adenovirus infected HeLa cells revealed patterns of regularly arranged particles in the nuclei. In further studies we have tried to correlate the development of specific intranuclear changes with the duration of infection.

In cultures infected with Type 5 virus ( $10^7$  ID<sub>50</sub> 10 cells) the particles and crystal like patterns were often observed to be adjacent to homogeneous grayish zones. The first distinct intranuclear changes appeared in some of the sectioned cells 24 hours after infection. Characteristic patterns were more frequent in later stages of infections (48 and 72 hours). At 72 hours when many of the cells examined showed almost complete disintegration and light microscopy showed cytopathic degeneration there still remained some cells showing a normal picture on electron microscopy. This suggested a lack of synchronization of virus production within the cells. It is also possible that not all cells participate in the production of new virus.

The suggestion that the intranuclear changes were specifically related to viral reproduction was strengthened by the positive correlation of their development with the rising titer of extracellular virus.

Concerning the morphology of the intranuclear particles rounded as well as elongated shapes were observed. The diameters ranged from 450 to 700 Å. Some of the elongated particles appeared to contain 2 round electron dense structures adjacent to each other.

At present we can offer no explanation for the grayish zones observed within the nuclei. However they seem to have some bearing on the development of the virus particles.

A full account of this work has been published elsewhere.

DR. MATTERN: In the 4 very interesting papers presented this afternoon we have heard described a variety of approaches to the study of several viruses. To those interested in the small animal viruses the 2 papers on the chemical and the physical properties of tobacco mosaic virus present a challenge namely that of catching up with the knowledge that has accumulated over more than 20 years of study of this plant virus. The paper presented by Dr. Schwerdt has indicated that this challenge is being accepted. It is along similar lines that our work at the National Institutes of Health is being directed. It is my privilege to discuss this work.

Coxsackie A 10 virus the etiologic agent of herpangina was grown in suckling mice and purified by methods previously described.<sup>6</sup> A virus pellet of high titer obtained by ultracentrifugation and covered with 1 per cent sodium chloride crystallized into dodecahedra. The formation of such crystals can be seen in Figure 213. In the upper portion of Figure 213 top left there is a portion of the ultracentrifuged pellet and at the periphery there is early crystallization. Figure 213 top right and bottom left and right shows continued crystallization of the same preparation with a concomitant disappearance of the amorphous material.

These crystals have obtained maximum dimensions of about 1/10 mm. They closely resemble the dodecahedral crystals of tomato bushy stunt virus and one strain of tobacco necrosis virus as obtained by Bawden and Pirie.<sup>1</sup> A somewhat different form of crystal was found when the purified virus pellets were covered with 5 per cent ammonium acetate. In Figure 214 top left they are seen as 8 sided plates. This was not the cleanest preparation we have had but it best shows the shape. Bawden<sup>1</sup> has described similar multiple crystal forms for two different plant viruses. On low speed centrifugation of such crystals it was found that about 99 per cent of the total infectivity was removed from the liquid phase.

Electron microscopy of crystalline preparations was performed utilizing the technique of replication. Because of the instability of these crystals on drying their replication—actually pseudoreplication—has not been entirely satis-

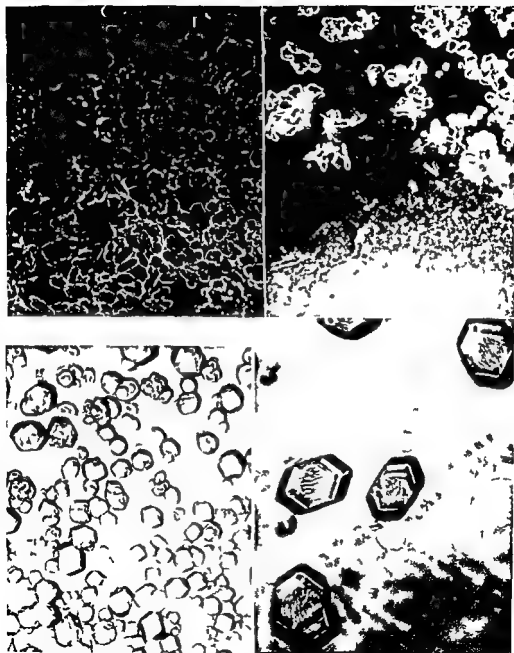


FIG. 213 (Top left) Portion of ultracentrifuged pellet at periphery early crystallization (Top right and bottom left and right) Continued crystallization of same preparation with concomitant disappearance of amorphous material

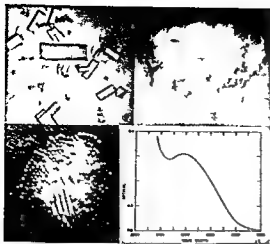


FIG 214 (Top left) Showing 8 sided plates (Top right) Showing hexagonal arraying at periphery (Bottom left) Similar aggregate with an area of rectangular arraying (Bottom right) Typical curve of nucleoprotein

factory. However both forms of crystal have shown large aggregates of approximately spherical particles 28  $m\mu$  in average arrayed diameter. Figure 214 top right shows such a preparation with hexagonal arraying at the periphery. The center of the mass is impenetrable to the electron beam because of multiple layers of virus. Figure 214 bottom left shows a similar aggregate with an area of rectangular arraying. The 3 angles in which the particles were found to array by electron microscopy were found to correspond to those angles measured on the crystal faces.

In attempting to determine the number of particles necessary to establish infection the volume of several crystal pellets has been estimated to be less than 1/100 ml. Such pellets have been found to have a titer of at least  $10^{14}$  LD 50's per ml. From the size of the particle it was calculated that about  $4.6 \times 10^{16}$  particles should be contained in a solid ml of virus. Thus it appeared that several hundred particles were necessary to establish infection by intra peritoneal titration of suckling mice. Intramuscular titration has resulted in a small increase in titer indicating that this ratio is likely to be close to 100 to 1.

The ultraviolet absorption spectrum in a solution of recrystallized virus was determined with

a spectrophotometer. A curve typical of nucleoprotein is seen in Figure 214 bottom right. Examination of the same solution in an analytical ultracentrifuge has revealed considerable inhomogeneity despite the ready crystallizability of the virus. Schlieren pictures have revealed at least two components. The major one with the sedimentation coefficient of about 150 Svedberg units corresponds to the virus particle. The other of about 80 units represents a major contaminant of reddish color. Companion pictures taken with ultraviolet light revealed the strongly ultraviolet absorbing virus component of about 150 Svedberg units and an additional component of about 40 units. It is of interest that Briefs and associates<sup>6</sup> purified the same virus from mouse carcasses by different methods and obtained the same sedimentation coefficient for the virus and also 2 contaminants of 40 and 80 Svedberg units. These contaminants have been largely removed by repeated ultracentrifugation and careful washing of the virus pellet to remove the reddish contaminant. The sucrose density gradient technic used by Schwerdt and Schaffer<sup>7</sup> should prove to be useful in removing such nonviral contamination.

Chemical analysis of this virus has been limited seriously by the small quantities of the purified material available. The virus was found to contain about 10.1 per cent total nitrogen by weight. This figure is somewhat lower than those reported for most plant viruses and bacteriophage (13.5 to 17%) but is within the range reported for a number of other purified animal viruses (7.7 to 15%). Utilizing the orcinol and indole tests for ribose and deoxyribose as described by Cenotti<sup>8,4</sup> equivocal results were obtained in which a small quantity of ribose was found but no deoxyribose within limits of the test.

Three attempts were made to analyze the virus for nucleic acid bases. In two tests qualitative results were obtained on hydrolyzing the nucleic acid by a technic described by Markham and Smith<sup>5</sup>. By means of paper chromatography spots corresponding in RF to guanine, adenine, cytidylic acid and uridylic acid were obtained. No thymine, its deoxynucleoside or deoxyribotide have been found. These preliminary data are suggestive evidence that this virus contains ribonucleic acid as has been definitely established in the case of the polio viruses.

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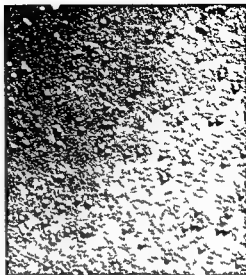


FIG 215 Electron micrograph of spherical virus particles obtained from human papilloma (Melnick *et al* *Ann New York Acad Sc* 54 1214)

Dr MELNICK Before taking up the main topic of my discussion I would like to show 3 electron micrographs of virus particles taken several years ago. The particles that were shown earlier today by Dr Schwerdt and by Dr Matern are hardly distinguishable morphologically from these. A purified preparation of the particles is shown in Figure 215. Like purified polio virus particles they tend to aggregate as shown in Figure 216 and even to form crystalline arrays as shown in Figure 217. When in crystalline formation the particles have a size of 57 m $\mu$ . As in other virus crystals the particles pack tightly in a hexagonal arrangement with connecting particles in adjacent rows making angles of 120° with each other. As indicated the slides are not new and they are not of polio virus particles. These preparations of human papilloma virus were obtained 8 years ago and it is gratifying to learn that other human viruses such as those of poliomyelitis and Coxsackie disease are now found to have a similar morphology and similar tendency to form crystalline arrays.

Ionizing radiation has been used successfully

by Pollard and others as a method for determining the internal structure of bacterial and animal viruses. A signal advantage of ionizing radiation methods over electron microscopy is the direct relationship which exists between the biologic activity of the unit and the experimental observations. There are advantages over ultrafiltration and ultracentrifugation in precision and in that one can determine by ionizing radiation what part of the virus unit is concerned with the function being studied.

In the irradiation procedures one measures the loss of biologic function resulting from damage done by ionizations within the unit involved. Particles with different densities of ionization along their tracks may be employed. Assuming random distribution of hits there will have been an average of 1 hit per sensitive unit at a point where 37 per cent of the virus survives.

Deuterons and alpha particles are relatively slow and when fired at the virus particle produce dense ionization along the track. The resulting inactivation is proportional to the area of the sensitive unit since at least 1 ionization may be assumed to occur in any virus particle through which a deuteron or alpha particle

may be more complicated. The ultrafiltration method gave a much larger size for this virus (140 m $\mu$ ) than did any of the irradiation methods (48 to 58 m $\mu$ ). Such differences are taken to indicate a differentiation of function with only the sensitive portion being uniquely essential. The relatively close agreement of the data for the measles sensitive unit obtained by electron bombardment with alpha particles and with deuteron measurements indicates that this sensitive unit is approximately spherical.

The size of the CF antigenic units of all of

the viruses studied fell into the same general range (8 to 13 m $\mu$ ). This is about the size of a moderately large protein. It seems to reside within the virus particle and obviously there is room for many of these CF units within the much larger virus.

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# General Considerations of Viruses

THURSDAY MORNING, JULY 11, 1957

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## *Chairman*

SIR MAC FARLANE BURNET

Royal Melbourne Hospital  
Melbourne

## *Speakers*

DR FRANK L. HORSFALL JR

Rockefeller Institute for  
Medical Research  
New York

DR MAX DELBRÜCK

California Institute of Technology  
Pasadena

DR KENNETH M. SMITH

Virus Research Unit  
Molteno Institute  
Cambridge, England

DR THOMAS FRANCIS JR

University of Michigan  
School of Public Health  
Ann Arbor, Michigan

## *Discussants*

*Moderator* DR ANDRÉ LWOFF

Institut Pasteur  
Paris

DR W. WILBUR ACKERMANN

University of Michigan  
School of Public Health  
Ann Arbor

DR WERNER SCHAFFER

Max Planck Institut für Virusforschung  
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DR RENATO DULBECCO

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## Opening Comments of Chairman

SIR MAC FARLANE BURNET

One of the problems which plagues me as it has many before me is whether or not it is possible to generalize about viruses at all. I have almost joined the majority who think that it is. Any type of virus multiplication involves a switch in protein synthesis by the infected host cell. From this point of view and from more superficial aspects of the necessity of transfer of a parasite from one cell to another one can legitimately find a basis by which a bacterial virus, a plant virus, an insect virus and a should we say vertebrate virus can be seen to have some common qualities.

Yet I cannot escape the feeling that C16-T2 which is the dominant species of intestinal bacterial virus and influenza A which is the most blatantly successful of human viruses are extraordinarily different things. For that matter so are vaccinia, influenza A and polio viruses.

Therefore it has been a difficult task for the designers of this program to provide what I think they had in mind. They wanted speakers who could touch on a wide enough range of topics to give us a sense that the problems of polio are merely a practically important area of an enormously greater field. It was necessary to remind us that bacteria, plants, insects and vertebrates all have their viruses and that in the interaction of parasite and host, of virus and susceptible cell there is a microcosm of the whole of functional biology.

The solution of those who developed the program is an ingenious one.

In this Congress animal viruses must have pride of place and two of our most distinguished animal virologists are to discuss one the multiplication of viruses and the other how

that multiplication can be inhibited. It is perhaps characteristic of our mid-century position that our concern here is not with the virulence of viruses and how the diseases and deaths that they produce can be prevented but with the search for an understanding of how our empirical successes in the practical field can be related to the basic concepts of biology.

By an accident of methodology and opportunity bacterial viruses tend to be considered in the same bracket as animal viruses while plant and insect viruses seem to have a similar illogical affinity. So we find Dr. Delbrück discussing the genetics of bacterial viruses perhaps the most elegantly logical field of our whole untidy subject. We shall find in that contribution a beautiful example of that special quality of the physicist and the mathematician that Dr. Delbrück more than anyone else has brought into virology. I do not despair of seeing a similar development in animal virology at the hands of Dulbecco, my former colleagues Fazekas and Cairns and the new generation.

I only regret that when it comes I shall be unable to understand it and that along with my older colleagues who came into virology when it was concerned only with preventive medicine I shall wonder sometimes just what is the bearing of it all on human affairs.

Finally Dr. Smith is to discuss the latency of viruses, a topic that has grown enormously in interest and controversy since Lwoff started looking seriously at lysogeny. Dr. Smith's examples will come from all the fields of virology and will be of special interest to those of us a majority I fancy who are not expert or knowledgeable in the plant and the insect viruses.

## *Viral Multiplication*

DR FRANK L. HORSFALL JR

The mechanism of viral multiplication has broad biologic implications for it is part of the more general problem of reproduction. It has wide medical implications too for unless viruses multiply in susceptible hosts virus diseases do not occur. The papers to be presented at this session of the Conference all will bear on certain aspects of viral multiplication; this is evident from their titles which include such terms as genetics, latency and inhibition. In the present discussion some attention will be directed to important areas in which evidence is inadequate or nonexistent and effort will be made to summarize what has been learned so far in other areas.

A virus particle is a tiny object with dimensions of about 250 millimicrons or less that can attach to and infect a susceptible host cell. Later in many instances similar objects leave the cell. In the interval between attachment and release multiplication occurs in the cell. The number of new particles that appear is usually larger than the number that attached to the cell and initiated reproduction.

The virus-infected cell is the unit of chief interest in relation to multiplication, not the virus particle or the host cell separately. This unit is in many ways a unique biologic complex with properties different from those of either partner alone and with new potentialities that cannot be recognized from the study of either separately. The most arresting capacity of the virus-infected cell is the production of new virus particles, a feat which neither partner can accomplish by itself. The process appears to involve initial disintegration of the infecting particle and in many instances is an unhealthy preoccupation for the host cell. Probably if viruses did not cause cell damage they would not have been recognized; moreover there would be no virus diseases nor any need for us to assemble in this Conference.

The major issue in relation to virus multiplication can be stated simply. How is the virus

particle reproduced in the infected cell? Apparently this is essentially a biosynthetic problem and a solution probably requires more precise and detailed knowledge of the biosynthesis of nucleic acids, proteins and other viral components than now exists.

With bacterial viruses much of the phage deoxyribonucleic acid (DNA) but little of the phage protein enters the bacterial cell. The important discovery of a new pyrimidine in phage DNA made possible studies on the intracellular synthesis of phage nucleic acid. As is now well known phage DNA is synthesized at a different rate than bacterial DNA. Recently it was demonstrated that phage DNA synthesis can proceed with little concomitant protein synthesis. The sequential synthesis of the two components indicates that phage DNA is not formed inside particles with protein membranes. In some as yet mysterious way the spermlike phage particles are finally assembled and are composed largely of DNA and protein, both of which are demonstrably different from those of the bacterial host cell.

With plant viruses there are now some strikingly similar indications. It was recently shown that ribonucleic acid (RNA) purposely separated from tobacco mosaic virus particles can initiate reproduction in susceptible plant cells. The close analogy to natural events in the initiation of multiplication of phage is fairly obvious. Unfortunately a distinctive marker of plant virus RNA has not yet been found so it has not been possible to follow its synthesis directly. The synthesis of TMV antigen, which is largely protein, can be followed but whether the antigen is a precursor of the virus particle or results from abortive biosynthesis is not yet clear. How the protein coat and the RNA are brought together to yield the final rodlike particles is still unknown.

With animal viruses there are now some indications of a similar kind. Recently published data indicate that ribonucleic acid preparations



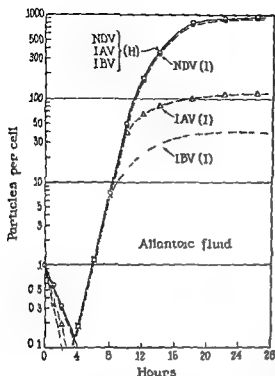


Fig 219 Growth curve of animal viruses

isolated from Ehrlich ascites tumor cells infected with Mengo encephalitis virus can initiate reproduction of that virus. The infective component it appears is the RNA itself and not residual contaminating virus particles. Thus bacterial, plant and animal viruses have achieved a certain unity for with each it seems probable that nucleic acid is the component that sets the stage for the production of new virus particles in the infected cell. Among animal viruses vaccinia appears to contain DNA but a number of others are now known to contain RNA. Identifying constituents have not been detected in the nucleic acids so their synthesis cannot yet be

followed directly. The production of soluble antigenic material which is mainly nucleoprotein can be traced but there is no decisive evidence that such soluble material is a precursor of the final spheroidal virus particles.

In contrast to the presently inadequate biosynthetic information there are many data on the dynamics of animal virus multiplication. Phage practice and theory have strongly influenced experimentation in this field and a number of findings are formally similar to those secured with bacterial viruses. Growth curves all tend to show comparable features and in semilogarithmic form closely resemble a ski jump as illustrated in Figure 219. They are flat at the bottom when postadsorptive and at the top and there is a steep slope in between.

To examine the kinetics of virus multiplication precisely it seems to be necessary to count virus particles. In the case of animal viruses this is in most instances a difficult problem (Table 119). With vaccinia particle counts by electron microscopy correspond closely to counts of infective units. With other animal viruses the correlation has been poor. The ratio of counted particles to infective units ranges from 5:1 with Newcastle, 35:1 with poliomyelitis and 100:1 with mumps to even higher values with other viruses. By definition the infective property is an essential attribute of a virus particle. Particles of similar form, composition and antigenic specificity which do not initiate multiplication are still of uncertain significance. Although they are clearly by-products of multiplication there is no evidence that they contribute or have much relevance to the process. Non-infective particles perhaps might be designated para-viruses; they are apparently dead-end objects which are unable to set the stage for reproduction.

TABLE 119 ELECTRON MICROSCOPE AND INFECTIVE UNIT COUNTS

VIRUS	COUNTED PARTICLES PER INFECTIVE UNIT	INFECTIVE UNIT PROCEDURE	REFERENCE
Vaccinia	0.7	Pock C.A.M.	Overman and Tamm 1956
Newcastle	5.0	Titration Allantoic	Isaacs and Donald 1955
Influenza	10.0	Titration Allantoic	Isaacs and Donald 1955
Fowl plague	10.0	Titration Allantoic	Isaacs and Donald 1955
Poliomyelitis	35.0	Plaque Assay	Schwerdt and Feoh 1956
Mumps	100.0	Titration Allantoic	Isaacs and Donald 1955

Approximate values

TABLE 120 PHOTOMETRIC HEMAGGLUTINATION AND INFECTIVE UNIT COUNTS

VIRUS	HEMAGGLUTINATING PARTICLES PER INFECTIVE UNIT	INFECTIVE UNIT PROCEDURE	REFERENCE
Influenza A	30	Titration Allanto c	Levine <i>et al.</i> 1953
Influenza A	11	Titration Allanto c	Horsfall 1954
Influenza B	16	Titration Allanto c	Horsfall 1955
Swine influenza	13	Titration Allanto c	Horsfall unpublished
Newcastle	18	Titration Allanto c	Horsfall 1955
Mumps	71	Titration Allanto c	Horsfall unpublished

Counts of hemagglutinating particles from erythrocyte sedimentation by photometric means (Table 120) show good correspondence to titration estimations of infective units with various influenza and Newcastle viruses but are poor with mumps. Such counts are based on a biological character much more distinctive than the particle morphology of animal viruses and give closely reproducible values. With influenza virus a fairly good correlation between them and electron microscope counts also has been secured.

Some of the difficulty in counting infective units is the striking instability of the infective property of various animal viruses (Table 121). The rates of thermal inactivation appear in most instances to be first order. The infective half-life at 35°C ranges from about 12 minutes with pneumonia virus of mice (PVM) and 85 minutes with mumps and influenza B to about 13 hours with polomyelitis and more than 24 hours with vaccinia. Studies on the dynamics of multiplications of unstable viruses are seriously affected by this feature and the yield time curves for infective units may be markedly different

from those determined with other particle characters.

Reproduction can be divided for purposes of analysis into 3 phases: latent period, increase period, and plateau. After attachment of the infecting virus particles to susceptible cells, most particles disappear and cannot be found. However, animal viruses penetrate into the host cell; it is not yet known though the possibility has been raised that those with a mucoproteinase like influenza may use this aid to enter. Disappearance may be due to disruption of the particle but good evidence for this has been brought forward only with influenza virus.

The usual assumption is that the necessary precursor materials are synthesized during the latent period and it may be that sequential biosynthetic processes are involved as seems to be so with phage. There is not as yet any direct evidence to support this idea but some indirect evidence which is not contrary has been secured with certain benzimidazole derivatives that inhibit RNA synthesis as well as with some amino acid analogues that inhibit protein synthesis.

TABLE 121 INFECTIVE HALF LIFE OF ANIMAL VIRUSES

VIRUS	ENVIRONMENT	INFECTIVE HALF LIFE	REFERENCE
		HOURS	
PVM	Moule lung suspension	0.7	Horsfall and Hahn 1940
Mumps	Allanto fluid	1.4	Horsfall unpublished
Influenza B	Allanto fluid	1.4	Horsfall 1955
Influenza A	Allanto fluid	2.4	Horsfall 1954
Swine influenza	Allanto fluid	8	Horsfall unpublished
Newcastle	Allanto fluid	14	Horsfall 1955
Polomyelitis	Tissue culture fluid	13	Foeh and Schaffer unpublished
Vaccinia	CAM suspension	>24	Orrman and Tamm unpublished

TABLE 122 LATENT PERIOD OF ANIMAL VIRUSES

VIRUS	HOST CELLS	LATENT PERIOD	REFERENCE
		Hours	
W E E	Chick embryo T C	1 2	Rubin <i>et al</i> 1955
Influenza	Allantoic membrane	3	Horsfall 1955
Influenza	Mouse lung	<4	Ginsberg and Horsfall 1957
Newcastle	Allantoic membrane	3	Horsfall 1955
Fowl plague	C A M culture	4	Schafer and Munk 1952
Poliomyelitis	HeLa	4	Ackermann <i>et al</i> , 1954
Poliomyelitis	Monkey kidney (single)	5	Lwoff <i>et al</i> 1955
Vaccinia	C A M	8	Overman and Tamm 1957
PVM	Mouse lung	15	Ginsberg and Horsfall 1951
Mumps	Allantoic membrane	30	Horsfall unpublished

The latent period (Table 122) ranges from 1 to 2 hours with Western equine encephalomyelitis and 3 to 4 hours with influenza. Newcastle and fowl plague up to 8 hours with vaccinia and 30 hours with mumps. In some different host cells influenza and poliomyelitis viruses appear to have nearly constant latent periods. New infective particles are not found in infected cells during most of the latent period regardless of how they are sought. Apparently the final assembly of new particles precedes their release from the cell by only a relatively brief interval.

The increase period shows in every instance so far examined a logarithmic phase. The kinetics correspond to that of an autocatalytic reaction and support the idea that one phase of multiplication is exponential. During the logarithmic phase the time to double the number of particles (Table 123) ranges from about 12 minutes with fowl plague and Western equine encephalomyelitis and 25 minutes with poliomyelitis to 8 hours with vaccinia and 15 hours with mumps.

The rate of logarithmic increase appears to be largely independent of the multiplicity over a wide range so long as the inoculum does not exceed about 3 particles per cell. When higher multiplicities are used the rate of increase of new particles may be reduced considerably particularly with influenza viruses. A similar reduction in the rate of increase may occur when a large number of noninfective particles are added to an infective inoculum that would itself give a relatively low multiplicity. With influenza and Newcastle viruses the hemagglutinating particle and infective unit counts correspond closely during the early hours of the logarithmic phase when the multiplicity is low. Apparently under these conditions most new particles are infective when they are released from the cell.

The release of new particles from infected cells in a number of instances seems to be gradual and continuous in contrast with the sudden release of phage on lysis of the infected bacterium. Release can continue for many hours after the concentration of virus particles has

TABLE 123 DOUBLING TIME OF ANIMAL VIRUSES

VIRUS	HOST CELLS	DOUBLING TIME	REFERENCE
		Hours	
Fowl plague	CAM culture	0.2	Schafer and Munk 1952
WEE	Chick embryo TC	0.2	Rubin <i>et al</i> 1955
Poliomyelitis	HeLa	0.4	Ackermann <i>et al</i> 1954
Influenza	Allantoic membrane	0.7	Horsfall 1955
Newcastle	Allantoic membrane	0.7	Horsfall 1955
Influenza	Mouse lung	2.5	Ginsberg and Horsfall 1952
Vaccinia	CAM	8.0	Overman and Tamm 1957
PVM	Mouse lung	8.0	Ginsberg and Horsfall 1951
Mumps	Allantoic membrane	15.0	Horsfall unpublished

During the logarithmic phase

reached nearly maximal levels in the infected tissue. The mechanism of virus release is still unknown but there are numerous indications from electron microscopy that the surface of the releasing cell does not appear to be entirely normal. To what extent filament formation as with influenza virus or localized cell surface lysis may contribute to the release of new particles is uncertain.

A concentration plateau often follows the increase period and with some viruses this may persist for many hours or some days. During the plateau period the concentration of virus particles may remain relatively constant even when measured by infective units. This may be the result of a near balance between continuing multiplication, release and inactivation of new virus particles. Because of the rapid rate of thermal inactivation of a number of animal viruses spontaneous inactivation *in vivo* can be an important feature in kinetic studies.

The yield of infective units per cell at the beginning of the concentration plateau (Table 124) ranges from approximately 600 to 2 000 with Newcastle, 100 to 1 000 with poliomyelitis and 50 to 500 with influenza viruses to about 10 in 50 with mumps.

A remarkable feature of the reproduction of some animal viruses is the effect of multiplicities greater than one on both the dynamics and the yield of infective units (Table 125). This seems to be most striking with influenza viruses including those in swine but is also evident with mumps and fowl plague although not with Newcastle. With multiplicities greater than one the rate of logarithmic increase is re-

duced and the yield of infective units may become very small. If the multiplicity is increased to high values the production of infective units may be almost completely inhibited. However noninfective particles which apparently are closely similar to the mature virus and cause hemagglutination although they lack the capacity to initiate reproduction are produced in about the expected large numbers. Closely similar results are secured also with input mixtures containing a low multiplicity of infective and a high multiplicity of noninfective particles.

The available evidence supports the idea that viral multiplication may be dependent on similar biosynthetic mechanisms whether the host cells are animal or plant including bacteria. Of special interest are the indications that the infecting virus particle loses its identity and is disrupted in the process of initiating reproduction in the infected cell. The concept that virus

TABLE 125. MULTIPLICITY AND INFLUENZA VIRUS REPRODUCTION

MULTIPLICITY OF SWINE INFLUENZA VIRUS	YIELD	
	HEMAGGLUTININATING PARTICLES PER CELL	INFECTIVE UNITS PER CENT
0.1	220	70
0.5	150	17
1.0	340	5
2.5	350	1.7
5.0	800	0.7
10.0	650	0.3

At 24-29 h. *in vitro*

TABLE 124. YIELD OF ANIMAL VIRUSES

VIRUS	HOST CELLS	APPROXIMATE YIELD INFECTIVE UNITS PER CELL	REFERENCE
Newcastle	Allantoic membrane	600-2 000	Horsfall 1955
Influenza A	Allantoic membrane	200-500	Horsfall 1955
Influenza B	Allantoic membrane	50-100	Horsfall 1955
Swine Influenza	Allantoic membrane	150-300	Horsfall unpublished
W. E. E.	Chick embryo T.C.	200-1 000	Dulbecco and Vogt, 1954
Poliomyelitis	Monkey kidney	300-1 000	Dulbecco and Vogt 1954
Poliomyelitis	Monkey kidney (single)	100-200	Lauff <i>et al.</i> 1955
Mumps	Allantoic membrane	10-50	Horsfall unpublished

At beginning of plateau

nucleic acid not the intact virus particle orients a number of biosynthetic events in the infected cell is a provocative and stimulating development in biologic theory. The indications that certain viral components particularly nucleic acid and protein are produced separately in sequential biosyntheses suggests that viruses are not reproduced as units but in a number of discrete steps. The final assembly of the new components into the completed virus particle remains a thoroughly mysterious process with the greatest enigma the acquisition of the capacity to infect another host cell. Recent work with some animal viruses raises the possibility that this may be controlled by something comparable to a feed back mechanism in that virus particles themselves in sufficiently high concentration can inhibit one of the last and perhaps one of the most subtle steps in the reproductive process: the development of infectivity.

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## Bacteriophage Genetics

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Originally I was asked to discuss the genetics of all types of viruses—animal plant and bacterial. At my request the topic was limited to bacterial viruses the only ones with which I am familiar from my own research. It would be more than hazardous to assume that what has been found for bacterial viruses should be true also for animal and plant viruses. These 3 classes of viruses are not tied together by any close bonds of relationship. Probably they are more closely related to their hosts than to each other. Their dissimilarity has found its most eloquent expression in the observation that the bacterial viruses now seem to be the only class whose genetic material is DNA both plant and animal viruses (at present accessible to genetic studies) being devoid of DNA. Presumably the plant and the animal viruses utilize their RNA for the storage of genetic information. There is certainly little point in extrapolating from one of these nucleic acids to the other.

Among the bacterial viruses themselves much diversity exists. The most distinct species and the most ubiquitous is the T2 C16 species. It has a speciality in its DNA that is absolutely unique: the base cytosine is missing; instead it contains hydroxy methylcytosine (HMC) and this HMC may be glucosylated at the hydroxy position. In some strains of this species all the HMC is glucosylated; in others only a fraction. In some strains the glucosylated fraction actually has diglucose attached. Most of the work on phage genetics has been done with representatives of this eccentric species particularly with the strains T2 and T4 and one might question the relevance of these findings even for other species of phages. Fortunately such questions can be answered clearly. Two other viruses have been studied in enough detail to show their essential similarity in respect to genetics to the T2 C16 species. These two other viruses are T1 studied by Bresch and his students in Göttingen and phage lambda a temperate phage studied by Jacob and Wollman at the

Institut Pasteur and by Kaiser at the California Institute of Technology. In addition some other phages have been studied less extensively.

Thus what I say makes no claim as to applicability to animal and plant viruses but does claim to be relevant for all phages irrespective of the peculiarities of their DNA and irrespective of their temperateness or virulence.

Basic for doing phage genetics is the ability to distinguish genetic types and in phage this is done almost exclusively by looking at phage colonies: the so-called plaques. Such a plaque contains a large number of phage particles but it is initiated by a single particle and the properties of this single particle determine the type of colony. Figure 220 illustrates a variety of colony types. They differ in diameter sharpness of edge turbidity and even in color in the technique used by Bresch. The type of colony depends also on the environmental factors during the formation of the colony such as the temperature the nutrient conditions the bacterial strains employed the dyestuffs added to the medium etc. These are chosen to suit the case to bring out the sharpest contrast between the types of colony produced by the various types of phage. Sometimes mutations occur during the growth of the colony giving a mixed or mottled appearance to the plaque and this mutability also may be used as a characteristic of the type of phage initiating the plaque. In some cases with which we will be concerned particularly the mottled appearance of the plaque is not due to mutations but is due to the fact that the original particle is duplex containing 2 opposing genetic elements which segregate on replication of the particle initiating the plaque (Fig. 221). Such phage particles with genetically mixed types are called heterozygotes and they will play an important part in our discussion. Principally we must keep in mind that by inspecting the plaques that they produce we can enumerate in a given sample of phage how many particles belong to each



FIG 720 Various plaque type mutants in a phage stock (T4) grown under conditions forcing the incorporation of 5 bromouracil instead of thymine into the DNA

type. Note particularly that in phage one characterizes practically every infectious particle in the sample because the probability that a particle will form a plaque is under proper conditions always close to 100 per cent.

The next point is the source of these various phage types. These may be either simply different types collected from various natural sources or types derived by mutation from one standard or wild type. For the experiments on recombination the results are ever so much simpler if we use a standard type and a group of mutants derived by mutation from it. The crosses between types collected in nature are by no means devoid of interest. Particularly the crosses between T2 and T4 studied by Streisinger and Weigle are fascinating because the parental types differ in the degree of glucosylation of the DNA. Thus we can study in these cases the inheritance of the chemical characteristics of the genetic material itself. These studies reveal some unorthodox features but they are as yet too incomplete to warrant discussion. When mutants are used in phage genetics those are in all cases spontaneous mutations. A special chemical compound 5 bromouracil an analogue of thymine has recently been shown to be highly mutagenic in phage. Litmann and Pardee have shown that phage grown on bacteria which are forced to use this compound instead of thymine to build DNA produce a prodigious amount of mutants. Figure 720 which I use to illustrate various phage types is in fact a picture of a 5 bromouracil culture. The mechanism of this



FIG 721 Mottled plaques. Such plaques may be due to (a) a phage with a high mutation rate (b) a mixedly infected bacterium (c) a phage heterozygote

mutagenesis is under intensive study as yet quite incomplete. Since the mutants so produced have not been used in any of the studies of phage genetics they need not concern us further.

Figure 722 illustrates a type of experiment that can be done. It shows the basic material for studying recombination. Bacteria at a high concentration are mixed with a fivefold excess of 2 types of phage the parental types. The phage particles attach themselves at random to the bacteria and after 5 minutes most of them are attached. Each bacterium has then been infected on the average with 5 particles of each parental type. The actual number varies of course from bacterium to bacterium but relatively few bacteria will not have been infected at all by one or the other or even by both parental types. After this adsorption period various dilutions are made perhaps including one dilution step through a tube containing specific antiserum to eliminate unadsorbed phage and the final dilution is calculated in advance to be such that a small measured volume contains on the average one fifth of one bacterium. A hundred of such small measured volumes are then distributed 1 to each tube. Most of these tubes will not contain bacteria or phage. About one fifth will contain 1 mixedly infected bacterium and a much smaller fraction will contain 2 or more. These tubes are incubated until lysis has occurred. At that time the tubes that contained 1 mixedly infected bacterium will now contain the yield of phage produced by this bacterium. The tubes then are plated and those that contained a phage yield will after incubation give a plate with plaques. 1 plaque for each phage particle in the progeny. These plaques are classified according to type and thus the plate tells



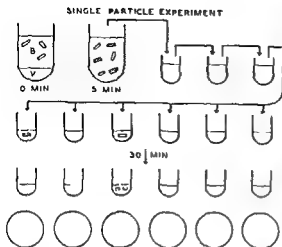


FIG 222 Schematic diagram of a single burst type experiment

us how many phage particles of each type are produced by this particular bacterium. The experiment here described is called a single burst type experiment and specifically we have been studying by this technic a genetic cross between the 2 parental types and more specifically we have made the cross under conditions of equal multiple infection (multiplicity 5 for each parent)

The principal findings that have been made by such crosses are the following

1 If the parental types differ by 1 mutational step then the yield contains both these types and no others. An exception is the case of phage T3 and its mutants where crosses between 2 parental types differing by only 1 mutational step give a great variety of types in the progeny. Obviously in this case the mutational event is more complicated. We do not know for certain why this is so and the speculations that have been made to account for this abnormality center around the interactions between phage genetic material and bacterial genetic material (See p 346)

2 If the 2 parental types differ by 2 mutational steps 1 affecting the size and 1 affecting the turbidity of the colony then the yield contains in addition to the 2 parental types the 2 recombinant types as shown in F

The proportion in w	= reco	up
may be as	r cent	60
per cent fo	types	ses

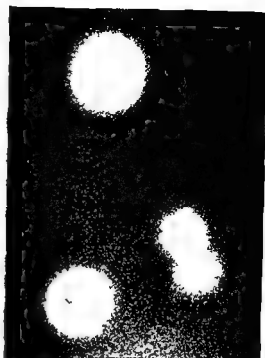


FIG 223 Four plaque types obtained in the progeny of a cross between two mutants (T2r and T2h) of phage T2. Besides the parental types T2r (large turbid) and T2h (small clear) there are present in the progeny also the two recombinants T2r+h+ (small turbid) and T2rh (large clear)

the proportion of recombinants may be as low as 01 per cent

3 The proportion of recombinants is characteristic for each pair of mutants and it constitutes the principal tool for constructing the genetic map of the phage and its mutants. The genetic map as such is not a matter of speculation but simply a concise summary of a large number of experimental results. The procedure for arriving at the map may be outlined as follows. Assume that we have collected a large number of mutants of a certain standard type of phage. We cross these mutants pairwise in all possible combinations and note for each cross the recombination frequency.

Then we note first that the mutants may split up into several linkage groups. Pairs belonging to the same linkage group have recombination frequencies less than the maximum for the

cross in question while pairs of which the members belong to different linkage groups all have the same maximal recombination frequency. The different linkage groups cannot be ordered in relation to each other on any kind of genetic map. They sometimes are referred to as the different chromosomes of the phage but this may well be an improper analogy.

Then we turn to the individual linkage groups and establish their genetic maps. We will describe the mapping procedure in a somewhat unconventional form to bring out what we think is the most essential part namely linear order without introducing the notion of map distance. We start with 3 mutants A B and C and look at the recombination frequencies for the pair AB for the pair AC for the pair BC. Let us say that the pair AC has the largest recombination frequency. Then we must put the mutant B on a straight line somewhere between A and C. Now we adjoin to the system a fourth mutant D and follow the same placement rule for each of the 3 triplets formed by combining the new mutant with each of the 3 previous pairs.

Thus we proceed adjoining 1 mutant after the other to the system and obtain in the end all the mutants arranged in an ordered series. Figure 274 illustrates this procedure for large distances. The fundamental fact is that such placements never contradict each other. For instance we never find that mutant D according to 1 triplet lies between A and B and according to another triplet lies between B and C while B lies between A and C. This is what we mean by saying that we can construct a linear map. Note that this definition does not involve the assignment of map distances.

In ordinary genetics one proceeds similarly but then finds in addition that the recombination frequencies also can be used to define map distances. That means that there exists a simple additivity rule. If B lies between A and C then the recombination frequency for the mutants A and C is roughly equal to the sum of the recombination frequencies for the pairs AB and BC. This additivity rule holds for ordinary genetics only for small distances. For larger distances it is disturbed by the occurrence of multiple recombinations and by the fact that these multiples may not be occurring according to simple statistical rules. In phage genetics the

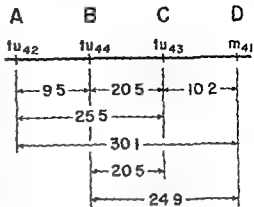


FIG. 274. Portion of the genetic map of phage T4 (Doermann A and Hill R. *Cenetus* 38:79).

additivity rule is disturbed by two further complications. First it has been shown that the phage cross does not correspond to a simple genetic cross. Inside a mixedly infected bacterium there occur quite a large number of recombinational events. In fact we are dealing with a small population somewhat analogous to the situation in ordinary genetics when we look at the progeny after several generations. Another complication which is prominent in phage genetics for small distances will be discussed later. The facts and notions here described were put together several years ago into a comprehensive formalism whose premises may be listed as follows.

The parental phage particles on infection of the bacterium enter a noninfective so-called vegetative stage. In this state they multiply and mate. The mating is random with respect to partner. If two genetically different vegetative partners mate a genetic recombination may occur and the probability that the recombination occurs during the mating act is some function of the distance between the loci assigned to the mutants on the genetic map. Specifically we assume that the probability of recombination increases with the distance on the map. The totality of the vegetative phage particles is called the pool of vegetative particles. From this pool particles are withdrawn at random beginning about midway between the time of infection and the time of lysis and the withdrawn particles become mature infective phage particles.

The maturation process is an irreversible process: mature particles never return to the vegetative pool. Also, they do not multiply and do not mate. They are quite inert.

The justification for this picture has been given in an excellent survey by Doermann and there seems to be no need to go over it again in this report since none of the more recent data lead one to question its validity. Great effort has been expended to nail down the physical nature of the vegetative phage particle. Is it or is it not simply naked DNA in the form known in extracellular phage? The T2 C16 species is advantageous for such studies because in this case HMC is a natural marker which distinguishes phage DNA from bacterial DNA. Moreover, the phage DNA synthesized in any particular time interval can be labeled differentially by introducing the radioactive phosphorus isotope P32 for a short interval into the medium. Moreover, DNA synthesis can be segregated from protein synthesis by suppressing the latter by various means. DNA thus synthesized in the absence of protein synthesis is incorporated later into phage after removal of chloramphenicol and resumption of protein synthesis.

These experiments demonstrate a separateness of DNA synthesis and protein synthesis and strengthen the idea that the vegetative phage is DNA, since this is the principal phage component injected into the bacterium upon infection and the only component which is transmitted in the progeny. Beyond this point the physical picture is less certain. It was believed earlier that RNA was not involved in phage replication because the RNA seemed to be metabolically inert, but now more refined methods have revealed a very small amount of rapidly turned-over RNA and this could be connected with DNA replication. Moreover, a variety of lines of evidence has led to speculation that the DNA of a phage may be bipartite, the two parts being functionally and structurally distinct. These problems have been reviewed in great detail recently and will not be treated here except to state that they still await clarification.

Returning to the formal picture of phage genetics developed above, we note that it leaves several questions of the most fundamental importance unanswered and we will devote the rest of this report to a statement of these ques-

tions and to the as yet largely unsuccessful attempts to answer them. Foremost is the question as to the interrelation between replication and mating. Here we may distinguish 3 notions which have been advocated.

1 Replication and mating are quite separate processes: recombination is a physical exchange between 2 structures not in the process of replication. This notion was implied in the theory of Visconti and Delbruck.

2 Mating always involves replication. The new replicas recombine in the process of formation. Besides this form of replication during mating, there is also ordinary replication not involving mating. This notion receives support from the structure of heterozygotes as will be seen.

3 Replication cannot occur except when 2 particles are mating.

Here we need not go into the numerous arguments that have been advanced in favor of or against these 3 alternatives. Suffice it to say that in our opinion none of the arguments are very strong. Perhaps a word of explanation should be added with respect to the last alternative. At first sight this seems to be absurd. How could replication be restricted to the mated state? In that case, single infection should never give any replication and those phage strains which show very little recombination should show very little growth. Of course neither of these is true. The supplementary hypothesis which has been suggested is that the genetic material of the host bacterium is sufficiently homologous to permit mating and in some cases even recombination of the phage with the host genome. Indeed, there is very good evidence in some systems of strong interactions and even recombinations between phage and host bacterium, but this evidence is by no means conclusive in showing that mating is a necessary condition for replication. These questions are discussed very fully in a review article by Stent.

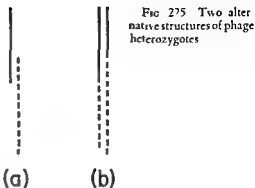
Closely related to the alternatives cited is the question whether the recombinational act produces just 1 recombinant or 2 and whether or not the parental types involved in the mating are preserved. We can list the alternatives a little more clearly in this manner: does the mating produce

- (a) 1 particle (a recombinant the parentals being destroyed)
- (b) 2 particles (reciprocal recombinants the parentals being destroyed)
- (c) 3 particles (2 parentals and 1 recombinant) or
- (d) 4 particles (2 parentals and 2 reciprocal recombinants)?

We know perhaps only one thing for certain regarding these alternatives and that is that none of them is quite right. The point which is wrong is the idea that the mating produces only parentals and recombinants. What it seems to produce instead is something less simple—a heterozygote and these we must consider now before we return to the stated questions.

First let us describe the phenomenon. Among the progeny of mixedly infected bacteria one finds besides the genetically pure particles also genetically mixed particles as shown by the fact that the plaques which these particles produce contain phage particles of two genetic types. It has been demonstrated beyond doubt that these mixed particles are not simply two particles stuck together but single particles containing in duplicate at least the particular section of the genetic map responsible for the type difference: one copy derived from one parent and one derived from the other parent. With respect to this section the particles are therefore heterozygous.

We know for sure two more things about the structure of heterozygotes: first the heterozygous section is short compared with the whole length of the genetic map; second outside the heterozygous section the particles are recombinant that is on one side of it they are pure for the genetic material derived from one of the parents and on the other side of it they are pure for the genetic material derived from the other parent. Perhaps we can add one more statement though not with quite the same degree of certainty. The frequency with which the heterozygotes turn up is compatible with the idea that for distant markers every recombination act produces in the first instance a particle with a short duplicate section somewhere in between the two recombined markers. Does this information suffice for writing down the complete genetic structure of the heterozygote? Unfortunately it does not. It leaves open the two alternatives shown in Figure 275. On the left



(a) the particle is described by a map which involves 2 partial replicas 1 of 1 parent and 1 of the other parent with a short region of overlap. On the right (b) the same particle is described by a duplex structure running the whole length of the genetic map. The portions outside the heterozygous region are homozygous representing the genetic material of 1 parent on 1 side and of the other parent on the other side. From a purely genetic standpoint we would not know how to distinguish the 2 structures because we have no way of predicting how either of them would behave. From a physical standpoint the principal difference is this: the structure *a* is duplex only for a short stretch and thus inhomogeneous along its length while the structure *b* is duplex along its entire length and thus physically quite homogeneous along its length. One might imagine that it should be easy to distinguish between these 2 alternatives by radiologic methods. One might expect perhaps that structure *a* should be inactivated like a single unit exhibiting a 1 hit curve and structure *b* like a double unit exhibiting a 2 hit curve.

Now it is certainly true that radiologically phage particles behave as single units but this does not really decide the point because we do not know how intimately the 2 hypothetical units might be associated. As a matter of fact we are certain that the genetic material in the mature phage is duplex at least in the sense of the Watson-Crick double helix. Therefore this duplicity at least does not show up radiologically. We could well imagine that there are even more complex duplex structures which do not show up radiologically. Thus in all we cannot decide between structures *a* and *b*. Of course we might permit ourselves to proceed specula-

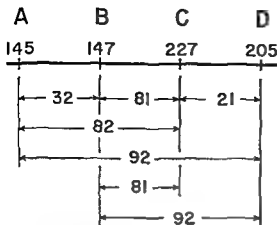


FIG. 226. Portion of the map of the *rII* region of phage T4 (Doermann Chase and Edgar)

tively and see which structure lends itself better to the construction of mechanical models aimed at describing mating heterozygote formation and recombination. However this approach has not been successful in narrowing down our choice.

We will drop the heterozygote story momentarily at this point but will bear in mind its principal inference that the mating act produces in the first instance a vegetative phage particle involving a short overlap region. Thus we cannot speak of the point on the genetic map which is involved in a recombination act. Instead we must speak of 2 points, the boundaries of the overlap region which belong to 1 recombination act. If the 2 parental types happen to be genetically different within this overlap region we wind up with a phage heterozygote. If the parents happen not to be different within the overlap region but outside of it then we wind up with a recombinant giving no evidence of its overlap region.

Let us turn now to the more recent studies of genetic fine structure initiated by Benzer who devised a special and very powerful technic for the study of genetic recombination in those cases where the recombination frequency is very low. The essence of this technic consists in the use of an indicator strain which does not propagate either of the types but is fully sensitive to 1 of the 2 reciprocal recombinants. Plating on such an indicator strain the progeny of a cross

permits one to detect and to count accurately 1 of the recombinants in the presence of an enormous excess of parental types. The work of Benzer and that of the Doermann group which we will describe presently concerns one particular short region of the genetic map of phage T4. However the same technic can be and has been applied to other regions.

The work of Benzer concerns the *rII* region of phage T4. The letter *r* designates a class of mutants producing large plaques on the usual host of this phage, this host being strain B of *E. coli*. Benzer isolated over a thousand of these *r* mutants and discovered that a large proportion of them have the property in common that an other strain of *E. coli*, strain A, is not lysed by them. These mutants infect the bacterium but shortly after that the processes leading to phage multiplication come to a standstill. The *r* mutants which do not form plaques on A constitute the class *rII*. They are by no means all identical. On the contrary, if two of them are crossed the chances are that they will form recombinants with a frequency ranging from 01 per cent to about 6 per cent.

Benzer has made a very elaborate study of these mutants and the first step of this study consists in mapping. It turns out also that in this case the linear mapping can be carried out consistently as illustrated in Figure 226. An exception is constituted by a small class of *rII* mutants which behave with respect to recombination as if they covered an appreciable section of the *rII* region. These do not form recombinants with any *rII* mutants in the covered section. These sectional mutants have also another property in common. They do not back mutate to the standard type, an indication that the mutational change has been more severe than in the case of all the simply mappable mutants. Among these latter there are subgroups which seem to be strictly isoclass. They form no recombinants with each other and this no really means no. The resolving power of the method for the detection of recombinants is so great that it can detect them when they occur a hundred times more rarely than the 01 per cent actually observed as the smallest finite value.

Benzer has found that the *rII* region actually consists of 2 contiguous portions A and B which control physiologically separate functions.

If a bacterium *K* is infected with two *rII* mutants then the infection proceeds normally if one is an *rIIA* mutant and the other an *rIIB* mutant but it is abortive if both belong to the *rIIA* class or if both belong to the *rIIB* class. This is a very important discovery in itself and it will be worth our while to linger over it for a moment. The experiments demonstrated in the first place that among the *II* mutants there exists a subgroup with the physiologic peculiarity of not being able to multiply on strain *K*. The mapping experiments then showed that this group of mutants also belongs together on the genetic map. They recombine with a maximum frequency of 6 per cent. Further they can be arranged among themselves on a linear map.

Next on the physiologic side again this group of mutants splits up into 2 subgroups such that members of the same subgroup cannot co-operate to bring about phage growth in strain *K* while any member of subgroup *A* can co-operate with any member of subgroup *P*. Next again on the genetic side it is found that the members of subgroup *A* occupy one segment of the genetic map while the members of subgroup *B* occupy a different segment which does not overlap with the first one but lies next to it. What this shows very clearly is that the genetic information is organized into functional units strictly 1 dimensional as far as genetic analysis can tell and that these functional units are arranged in tandem on the map as a whole.

Of course this is shown here only for two particular functional units and by no means should we jump to the conclusion that the whole of the genetic map is arranged as one long tape. It is entirely conceivable and in my mind even probable that for longer distances another organizational principle may supervene just as long manuscripts are not published as tapes but are broken up into lines and pages and volumes.

We mentioned before that Benzer found for his *rII* mutants that there exists a finite minimum recombination value about 0.1 per cent. A similar value was found for a different region of the genetic map by Streisinger and Franklin. The meaning of this minimum finite value is at present entirely a matter of speculation and shall not concern us here. However we will follow Benzer in his analysis one step further in the study of those sub-subgroups of mutants

which show zero recombination each with each. The members of such a group are then genetically speaking strictly isoclonal. Benzer studied particularly one such group comprising 123 members. The motive for undertaking such a study is that of wishing to find out something about the limits of variety that such a group of isoclonal mutants can exhibit. In other words how many shapes can one point on the genetic map have? Such a question of course comes quite naturally these days when we constantly have before our minds the picture of the DNA molecule as the physical bearer and repository of genetic information.

Now a point on this molecule is certainly not less though possibly more than 1 nucleotide pair. If it is just 1 nucleotide pair then presumably it cannot take more than 4 shapes corresponding to the 4 base pairs and thus our group of isoclonal mutants should not include more than 3 varieties the fourth being the unmutated variety. Among the 127 members of 1 isoclonal group mentioned above Benzer did in fact find 3 varieties and not more distinguishable by their rate of reversion to the wild type. So this looks very hopeful for the point of view which assumes that a point mutation is an exceedingly simple and narrowly circumscribed chemical change in a molecule whose structure is well understood. A detailed study of mutagenesis in phage with mutations produced by 5-bromouracil using Benzer's criteria for identification of the mutant should enable us to tell a great deal more.

With these ideas about genetic fine structure in mind let us now return to the problem of the mechanism of genetic recombination and of the role of the heterozygote in this mechanism. To begin with let us consider a cross between 2 *r* mutants belonging to the same or to different physiological subunits of the *rII* region. What are we actually measuring when we make such a cross and plate the progeny on the indicator strain *K*? In the first instance we will be counting to be sure true recombinants which are wild type with respect to both of these *r* locations. But what about the heterozygotes? We had arrived previously at the notion that the recombinational event produces in the first instance a particle containing an overlap region of appreciable length much longer than the minimum recombinational distance. Therefore

most of the primary products of recombinational events will be doubly heterozygous and some of them will be singly heterozygous. Will these heterozygotes register on K? Fortunately we know the answer to this question. They will register on K at least with appreciable probability if the 2 *r* mutants belong to different subunits but will not register when they belong to the same subunit. The fact that the heterozygote does register on K when the two *r* mutants belong to different functional subunits means that these heterozygotes simulate the 2 recombinants and that they have to be corrected for when one wants to determine the recombination frequency. This was not appreciated at first and made it appear as if there were a gap between regions A and B.

The fact that the heterozygotes will not register when the markers belong to the same subunit tells us something new about the heterozygote. It tells us that the functional unit fails to function not only when part of it is in one phage and part in the other phage but also if part of it is in one of the overlapping segments of the heterozygote and the other in its counterpart. That these heterozygotes which fail to register on K are potent producers of true recombinants can be shown very elegantly. If the original progeny of the cross is adsorbed on strain B before plating on strain K, the plaque count increases and this increase is due to the fact that most of the heterozygotes whose overlap region covers both loci now do register. They do so because they can function on B and during their 1 cycle of growth they need produce only 1 wild type recombinant to be able to continue to grow on K and to go on to produce a plaque. They do produce a wild type recombinant much more often than if the bacterium had simply been infected with one each of the parental types. This experiment shows perhaps more clearly than any of the others the pivotal role which the heterozygotes play for the recombination of closely linked markers.

For such pairs recombination occurs largely in 2 steps. In the first step a heterozygote is formed during a mating of the parental particles and the overlap region of this heterozygote covers for very close markers mostly both loci. In the second step a true recombinant is formed

However at this point we are still in the dark. We do not know what a heterozygote does when it replicates. Does it copy itself as heterozygote or does it produce only segregants i.e. non heterozygous progeny? If it does produce only segregants does it produce one at a time through several replications so that it can throw off several segregants and perhaps even several kinds of segregants? Or does it produce 2 segregants at a time and perish during the first replication act? These questions have not yet been answered with any degree of certainty but presumably will be answered soon because new techniques permit one to screen out the double heterozygotes and to study their progeny by single burst techniques.

Two rather qualitative things about the heterozygotes can be said.

1 A single factor heterozygote produces principally parental types and not copies of itself.

2 A double heterozygote involving 2 closely linked markers produces in addition to the parental types also recombinants and it produces these recombinants more frequently than a bacterium merely infected with the parental types. However we do not know whether this excess of recombinants is due to a direct production during replication of the heterozygote or to a more indirect mechanism as follows. The heterozygote first produces parental type offspring without however perishing in the act. The recombinants are then produced during subsequent matings between the heterozygote and its offspring.

Still other mechanisms involving the production of partial replicas and their transfer from one matrix to another have been discussed by Barricelli and by Bresch.

Intimately connected with this 2 step nature of the formation of recombinants between closely linked markers is the phenomenon of negative interference studied intensively by Doermann Chase and Edgar. The term interference is taken over from ordinary genetics in higher organisms. There it signifies the fact that recombination in one region interferes with the occurrence of recombination in an adjacent region. This interference is positive i.e. recombination in one region reduces the probability in an adjacent region. In phage genetics we find the opposite—negative interference i.e. recombi-

nation in one region increases the probability in an adjacent region. This was first discovered for phage recombinations involving fairly distant markers and was explained very simply and satisfactorily by taking into account that a phage cross involves several rounds of mating and that the particles in the progeny have not all mated with their opposite genetic types equally often.

Therefore by looking in the progeny at the class of particles which show recombination in one region we introduce a statistical bias in favor of those particles which have mated with the opposite genetic type at least once. It is therefore reasonable that we should find in this selected class more recombinants with respect to another linked region than in the total progeny. However Doermann and Chase discovered that there exists a much stronger negative interference than can be accounted for by this argument when short regions are involved. In these studies the mutants of the rII region were again used. By special techniques Doermann and Chase succeeded in isolating from crosses between  $r^-$  mutants not only the wild type recombinants but also the reciprocal recombinants carrying both the parental  $r$  markers. Using these double  $r$  stocks as parental phage a great variety of 3 or 4 factor crosses could be carried out. Let us symbolize the various  $r$  markers as a b c, the alphabetical order corresponding to their order on the genetic map. Numerous crosses of the following types were performed:

(1) $\frac{ab+}{++c}$	(2) $\frac{a+c}{+b}$	(3) $\frac{a++d}{+bc+}$	(4) $\frac{a+c}{+b+d}$
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To produce a wild type particle the first cross requires recombination between b and c and no recombination between a and b. The second cross requires 2 recombinations between a and b and between b and c. The third cross requires recombination between a and b and between c and d and no recombination between b and c. The fourth cross requires recombinations to occur in each of the 3 regions. The experiments of Doermann and Chase show very convincingly in all of these cases that the recombinational events in these short regions are strongly correlated and that this correlation effect is limited to regions of the length of the heterozygote overlap region. In view of what we have said (p. 340) about the 2 step nature of the production of recombinants when closely linked markers

are involved this finding is eminently reasonable in the present case when we select in the progeny a particle which is recombinant in one region we select not only one which must have mated with an opposite partner but also one which very probably in its ancestry must have had one member which was heterozygous for most of the region considered. Of course this introduces a strong bias in favor of other recombinations with the region. Perhaps it will be possible from the detailed quantitative studies to arrive at definite conclusions about the behavior of heterozygotes during subsequent replications.

The question whether reciprocal recombinants are formed *pari passu* or statistically independently must now be put separately for close in contrast with distant markers. For distant markers where the overlap region is not likely to cover a marker the question stands much as before with the additional specification that the reciprocal recombinants would have to contain reciprocal overlaps. The most detailed study is due to Bresch working with mutants of phage T1. The rationale of his experimental setup is the following. We know that in 2 factor crosses reciprocal recombinants are formed statistically in equal numbers when the yield is averaged over a large number of single bursts. However when one looks at individual single bursts one finds practically no correlation between the numbers of reciprocal recombinants produced. This lack of correlation might mean that the reciprocal recombinants are formed independently of each other or it might mean that they are produced pairwise in the first instance but that this strict correlation is not apparent in the single bursts because it is wiped out by two randomizing influences affecting the two recombinants independently. One of these randomizing influences is maturation, the other is replication after recombination. The latter influence can be reduced by disregarding the actual numbers of recombinants found in single bursts and scoring instead for presence or absence of a given recombinant. Such a correlation for the presence of reciprocal recombinants should be the more striking the rarer the recombinant, i.e. the closer the linkage between the markers. However it is not possible for this experiment to take 2 markers belonging to the same func-



tional unit because then only 1 of the reciprocal recombinants could be scored conveniently. Detection of the other the double mutant would require elaborate backcrosses. Thus close but not too close markers are needed.

Another precaution that must be used is that of avoiding a confusion of a correlation due to the suspected pair production of the reciprocal recombinants with a spurious correlation due to other causes. Principal among these spurious causes would be an inhomogeneity among a group of infected bacteria with respect to the total amount of mating taking place in each. If there are some in which much mating takes place and some in which little mating takes place this also would lead to a correlated appearance of reciprocal recombinants even if they are not produced in the same act.

In Bresch's studies this spurious correlation was corrected for by making 3 factor crosses instead of 2 factor crosses. In a 3 factor cross there are 6 recombinants and one can study the correlation not only between reciprocal pairs of recombinants but also between other pairs which are not reciprocal and therefore would not be expected to be made in one act. Indeed Bresch found a weak correlation between reciprocal recombinants but exactly the same correlation was found between nonreciprocal pairs and both correlations were ascribed therefore to spurious causes. Bresch's evidence then strongly favors statistical independence in the production of reciprocal recombinants.

However for close markers the situation is different. Here as we have said the recombinants are formed in 2 steps. The first step is often the formation of the double heterozygote but we must now focus our attention on the second step the formation of recombinants from the heterozygote. Are these produced singly or as reciprocal pairs? To my knowledge this question has not yet been attacked experimentally but very probably will be settled in the near future.

Let us try to sum up to see where we stand. Genetic mapping has disclosed the existence of linkage groups within linkage groups a gross linear order and within short regions again a linear order closely correlated with the physiologic functions controlled by the genetic material. Further a minimum recombination fre-

quency has been found and strictly isosomal mutants have been found to display only a small number of varieties. These findings are compatible and in fact are in striking agreement with expectations if it is assumed that DNA molecules constitute the physical counterpart of the genetic information. However they do not demand that the entire map is to be thought of as one long DNA chain.

The picture of a phage cross as a series of matings in a small population of vegetative particles has been sustained. A major novelty is the conclusion that the recombinational act involves not a point on the genetic map but a finite region. The primary product is one particle containing an overlap region not a pair of reciprocal recombinants. In the overlap region both parental particles are equally represented. If the parental particles differ in this region a heterozygote results. Outside the overlap region the particles are genetically pure on one side of the overlap representing one parent on the other side the other parent. The physical nature of this overlap is uncertain. Specifically it is uncertain whether the particle is thicker in the overlap region than outside. For recombinations between closely linked markers the overlap feature becomes very important. A large proportion of the recombinants are formed in two successive steps the first leading to the formation of an overlap longer than the distance between a close pair of markers the second leading to the formation of the recombinant proper. The details of the behavior of the overlap region during subsequent replication and mating are still very much in dispute.

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# Viral Latency

DR KENNETH M SMITH

Here is a definition of latency as suggested to me by Dr Robley Williams. Latency is a nonspecific term indicative of our ignorance of what is transpiring

## INTRODUCTION

There is much confusion of thought regarding viral latency and opinions differ as to whether it consists of a special kind of virus or a special kind of host. Luria considers that viral latency is not a special situation but only an extreme one and that any virus must have some host in which a balance has been achieved whereby the continuation of the virus is ensured. Partial latency and by that presumably is meant latency in some hosts is almost a necessity for every virus.

I define viral latency broadly as the condition in which an organism is infected with a virus but shows no external evidence of the fact. That the virus is present means that the infected cells are internally different from uninfected cells. As seen from examples given later this fact is sometimes demonstrable directly by current techniques but in some examples of latency it is not.

The course of events leading to the condition of latency can be divided arbitrarily into 4 types. First there are viruses that cause evident symptoms when they first infect an organism; these symptoms disappear but may recur at intervals as the consequence of some stimulus. An example of this is herpes simplex in man.

Second there are viruses that also initially cause a disease but the host recovers and although it still contains the virus never again shows external evidence of doing so. Belonging to this category perhaps is the virus of lymphocytic choriomeningitis in mice referred to by Andrewes as an indigenous virus. Traub showed that the virus persisted in the blood of mice after recovery from infection and that female mice thus carrying virus could infect their young in the uterus. After the passage of 2 years the virus in the stock mice had become

milder and milder so that its presence could be demonstrated only by inoculation into a non-carrying strain of mice. This is a rare case in the field of animal viruses of the use of an indicator host. However in plant virus work the use of such indicators is one of the routine methods of detecting the presence of latent viruses. Several plant viruses can be put in this second category where an initial reaction is caused notably the tomato black ring virus and other viruses of the ringspot type. The criticism may be made that viruses of this second category are not strictly speaking latent because the host has gained the upper hand insofar as the virus persists but may not be able to multiply sufficiently to cause observable symptoms. However the fact that the virus persists and that symptoms are absent are sufficient to warrant inclusion under our definition of latency.

To the third category belong those viruses which apparently never cause a disease in their original host and cannot so far as I am aware be induced to do so. Of course the original host in this context is the host in which the virus was originally discovered. In this group are mainly plant viruses but the salivary gland virus of guinea pigs which does not cause a disease in the original individual may provide a comparable type of phenomenon in animals. Three plant viruses which seem to be truly latent in their original host are the paracrabapple virus of the King Edward VII potato, the latent virus of dodder *Cuscuta* sp. and a virus which occurs commonly in sugar beets and mangolds.

The fourth category may represent a fundamentally different condition: the original hosts are not known ever to have shown symptoms and they seem to be free from detectable virus until they are subjected to some appropriate stimulus when a virus immediately becomes detectable and usually kills the host. Examples of this type occur in insects and in the lysogenic bacteria. This is referred to again later.

There is some controversy as to whether

latency can be differentiated from masking or whether such a thing as masking exists at all Shope considers that the rabbit papilloma virus is masked in domestic rabbits and its presence can be demonstrated only with difficulty and by indirect means. However Beard disputes this and says that masking in tumor viruses can be explained on the basis of low titer alone. However this is not the case with the lysogenic bacteria where the phage appears to be in a different physical state. Some of the latent insect viruses also possibly may come under the heading of masking rather than of latency.

To summarize. The presence of a latent virus may be shown in 1 of 3 ways: either the host develops the disease spontaneously after a period of time or the disease can be induced by various methods of treatment or in order to see the virus at all it must be transferred to a new host.

## SOME EXAMPLES OF LATENT VIRUSES

### PLANT VIRUSES

With the possible exception of the insects more viruses apparently are latent in plants than in any other type of organism. Indeed it is probable that only a few of the viruses latent in weeds and other wild plants have been discovered yet.

Latent viruses have been detected in hops, strawberries, potatoes, raspberries, dahlias, weeds of various sorts, sugar beets, and even in giant trees of the tropical forests. It is becoming increasingly clear that there is a group of soil-borne viruses that cause ring-spot symptoms in some hosts and occur in a range of apparently normal weeds and other plants. When some of these viruses infect cultivated crops such as raspberries, lettuce, and tomatoes, they cause quite severe diseases and become of economic importance.

One of the most interesting of these latent plant viruses is that from the sugar beets previously mentioned as being in the third category of latent viruses. This virus usually can be found in the commercial sugar beets in the British Isles and other countries, notably the U.S.A. Although it occurs commonly in sugar beets, all attempts to find how it spreads have failed, and tests for insect vectors and for soil

and seed transmission have proved to be negative.

### INSECT VIRUSES

Latent viruses occur in abundance in the larval forms of insects, mainly the Lepidoptera. These are of the polyhedral type, and both the nuclear and the cytoplasmic kinds occur in the latent condition. There seems to be little doubt that large populations of insects exist in which the greater proportion carry a latent virus. This is particularly true of the garden tiger moth, *Arctia caja*, of the silkworm, *Bombyx mori*, and of the spruce budworm, (*Choristoneura fumiferana* [Clem.]) in Canada.

### VIRUSES IN HIGHER ANIMALS

To mention a few of the many latent viruses which have been discovered in higher animals: rabbits carry Virus III in the testes; mice have Theiler's encephalitis virus and the virus of lymphocytic choriomeningitis, previously mentioned. Carr says it cannot be emphasized too strongly that the majority of commercial stocks of fowls are naturally carriers of the viruses from an early age of the leukosis complex to which group the sarcoma and the leukemia viruses belong. Fowls are also capable of carrying the virus of fowlpox.

## DETECTION OF LATENT VIRUSES

### IN PLANTS

1. By Means of Indicator Plants. This method is dependent on finding a susceptible plant host which will react in an unmistakable manner to the virus latent in the plant under test. The discovery of such a plant is a matter of trial and error, although certain plant species do react clearly to an unusually large number of viruses. These include the French bean, *Phaseolus vulgaris*, the cowpea, *Vigna sinensis*, and certain chenopodiaceous plants like *Chenopodium amaranticolor* and *Gomphrena globosa*. For example, inoculation of the sap from sugar beets containing the latent virus previously mentioned produces on the inoculated leaves of cowpea characteristic local lesions within 4 days. The tobacco plant, *Nicotiana glauca*, is susceptible to infection with more viruses than any other known plant and so makes another useful indicator host.

2 By Serology Many plant viruses are excellent antigens and if partially purified and inoculated into a suitable animal such as the rabbit each gives rise to an antiserum which reacts only with that virus and related strains. This is a useful tool in plant virus studies and has a special application to the investigation of latent viruses. First it affords a rapid and delicate test for the presence of a latent virus and second it may reveal unsuspected relationships between latent viruses. The technic has been used recently by Kassanis in an investigation of 3 viruses which may occur latent in potato and carnation plants. By this means he has shown that a relationship exists between a latent virus which is insect borne and 2 which are not and thus has thrown light on a problem which has long been obscure. This matter is further discussed in the section headed Mutation and Latency in Plant Viruses.

Some of the more progressive seed potato growers now use the serologic test to detect the presence of the latent potato virus X so that they can select for propagation plants that are virus free. This virus is widespread in some potato varieties and even though it causes no observable symptoms it can still reduce the yield by as much as 10 per cent.

3 By Electron Microscopy This method is still only of academic interest since the labor of searching for a latent virus in a plant cell is considerable unless the virus is known to be of a characteristic and easily recognizable shape. It has perhaps a limited application in the cases of rod shaped or filamentous viruses such as that of a latent tobacco mosaic or potato virus X but these are detected more easily by other means.

#### IN INSECTS

Latent viruses affecting the larval stages of insects are perhaps most easily detected by various methods of stimulation and this subject is discussed at greater length in the Section headed Stimulation of Latent Viruses.

Not much work has been done on the electron microscopy of latent insect viruses but there is one case on record of what possibly might be a latent virus revealed by this method. In the blood of apparently normal house crickets (*Gryllus domesticus*) masses of viruslike particles were observed.

#### IN HIGHER ANIMALS

Various methods are available for detecting the presence of latent viruses in the higher animals. The salivary gland virus of guinea pigs was detected first by means of intracellular inclusions observed on the optical microscope.

Stimulation by different means is another approach to the problem while the most efficient method is probably by serologic reactions.

#### STIMULATION OF LATENT VIRUSES

##### BY SERIAL PASSAGE

Serial inoculation in animals sometimes leads to the development of symptoms presumably because the latent virus has increased in virulence or has been stimulated to multiply more vigorously. For example two latent viruses in mice can be brought to light by this method. Progressive inoculation with lung extracts frequently produces typical pneumonia from which a pneumonia virus of mice can be isolated. Similar serial transmission stimulates into activity Theiler's mouse encephalitis virus.

Little information is found on the effect of the inoculation of body fluids in progressive series through insects though it is probable that a latent virus might easily be activated by this procedure.

Neither is there precise information on the effect of serial passage on latent plant viruses but presumably a tolerant host would continue to be a tolerant host after serial passage through the same plant species. In this connection serial passage of some viruses not necessarily latent through unusual plant hosts produces an apparent change of virulence which usually is due to a selection of one virus strain from a mixture of strains. Possibly during serial passage of a latent plant virus a second latent virus could be picked up on the way when the combination might produce observable symptoms. There are instances in some plants potatoes for example of more than one latent virus existing together.

##### BY THE USE OF ARTIFICIAL IRRITANTS AND CHEMICALS

Many latent viruses can be stirred readily into activity by artificial stimulations of different kinds. A carrier of the Rous I fowl sarcoma reacts violently to injection with methylcholanthrene resulting in the formation of a Rous I

sarcoma. In the Shope papilloma virus in both domestic and cottontail rabbits applications of tar cause the papillomas to become malignant. Here presumably the action is in changing the response of the cell to the virus. Similarly in fowls carrying the virus of fowlpox the virus can be awakened by painting with hydrocarbons. Here again the disease progresses much beyond the normal until a malignant growth develops but identical experiments carried out on fowls free of the virus failed to have any effect.

Andrewes points out that the virus of mouse hepatitis apparently exists as a purely latent infection of many stocks of mice. However unlike some latent mouse viruses serial transmission does not stimulate the hepatitis virus into action. The equilibrium can be upset by the injection of another parasite *Eperythron coccoides*. This appears to activate the virus in some way which becomes evident after further serial passage.

The work of Yamafuji has shown that apparently normal silkworms (*Bombyx mori*) develop polyhedral virus disease after feeding on foliage contaminated with some chemicals such as nitrates. Yamafuji's interpretation of this is that the virus is produced *de novo* but in view of the very high incidence of latent polyhedral viruses in lepidopterous caterpillars the alternative hypothesis of stimulation of a latent virus seems to be preferable.

The lysogenic bacteria represent a different situation from that of most latent viruses. The virus seems not to exist as particles such as occur *in vitro* for infectious phage cannot be liberated by the mechanical breaking open of the lysogenic bacteria. Its state is indicated by the name prophage and it must undergo some kind of maturation process before it becomes virulent. However it is possible to induce mass lysis and the liberation of infectious phage by certain kinds of artificial stimulation notably ultraviolet light and treatment with hydrogen peroxide.

#### BY INOCULATION WITH A FOREIGN VIRUS

Some good examples of virus stimulation by inoculation with a foreign virus have been found in insects. To understand this it is necessary to mention briefly the different types of insect viruses. First there are the polyhedroses in

which certain tissues become filled with virus containing polyhedral crystals (there are 2 types of polyhedral diseases the nuclear and the cytoplasmic in which the viruses multiply respectively in cell nucleus or cytoplasm) second there are the granuloses in which the tissues become filled with minute granules containing the virus usually a single rod shaped particle per granule and third there are the diseases in which the virus is free in the tissues without intracellular inclusions.

Preliminary cross inoculation studies have shown that inoculation with a nuclear polyhedral virus frequently will stimulate into action a latent cytoplasmic virus. This may have practical significance. There is an effort being made to use viruses in the control of certain insect pests. We have been experimenting with a certain rather virulent pest but we could not find the virus. So we gave it a dose of a virus of a different kind from the larvae of a butterfly and in one experiment it produced 100 per cent mortality but with an entirely different virus. In other words the nuclear virus which we fed to the caterpillar invoked a latent virus which apparently did not occur in nature. By that I mean that we have never been able to find in the insect the naturally occurring virus diseases of its kind but having once stimulated the cytoplasmic virus we could then pass it on indefinitely in series. Search for a naturally occurring virus in the larva of the winter moth *Operophtera brumata* has failed but inoculation of apparently healthy winter moth larvae with a foreign nuclear polyhedral virus from the larvae of the painted lady butterfly *Ianessa cardui* produced a high mortality. Examination of the dead winter moth larvae showed that they had died from a cytoplasmic polyhedrosis. Once stimulated this virus was indefinitely transmissible in series. At the moment observations appear to be lacking as to whether or not the reverse phenomenon is true i.e. whether or not inoculation with a cytoplasmic polyhedral virus will stimulate a latent nuclear polyhedrosis. Apparently a granulosis virus cannot stimulate a latent polyhedral virus or vice versa.

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2 By Serology Many plant viruses are excellent antigens and if partially purified and inoculated into a suitable animal such as the rabbit each gives rise to an antiserum which reacts only with that virus and related strains. This is a useful tool in plant virus studies and has a special application to the investigation of latent viruses. First it affords a rapid and delicate test for the presence of a latent virus and second it may reveal unsuspected relationships between latent viruses. The technic has been used recently by Kassanis in an investigation of 3 viruses which may occur latent in potato and carnation plants. By this means he has shown that a relationship exists between a latent virus which is insect borne and 2 which are not and thus has thrown light on a problem which has long been obscure. This matter is further discussed in the section headed Mutation and Latency in Plant Viruses.

Some of the more progressive seed potato growers now use the serologic test to detect the presence of the latent potato virus  $\lambda$  so that they can select for propagation plants that are virus free. This virus is widespread in some potato varieties and even though it causes no observable symptoms it can still reduce the yield by as much as 10 per cent.

3 By Electron Microscopy This method is still only of academic interest since the labor of searching for a latent virus in a plant cell is considerable unless the virus is known to be of a characteristic and easily recognizable shape. It has perhaps a limited application in the cases of rod shaped or filamentous viruses such as that of a latent tobacco mosaic or potato virus  $\lambda$  but these are detected more easily by other means.

#### IN INSECTS

Latent viruses affecting the larval stages of insects are perhaps most easily detected by various methods of stimulation and this subject is discussed at greater length in the Section headed Stimulation of Latent Viruses.

Not much work has been done on the electron microscopy of latent insect viruses but there is one case on record of what possibly might be a latent virus revealed by this method. In the blood of apparently normal house crickets (*Gryllus domesticus*) masses of viruslike particles were observed.

#### IN HIGHER ANIMALS

Various methods are available for detecting the presence of latent viruses in the higher animals. The salivary gland virus of guinea pigs was detected first by means of intracellular inclusions observed on the optical microscope.

Stimulation by different means is another approach to the problem while the most efficient method is probably by serologic reactions.

#### STIMULATION OF LATENT VIRUSES

##### BY SERIAL PASSAGE

Serial inoculation in animals sometimes leads to the development of symptoms presumably because the latent virus has increased in virulence or has been stimulated to multiply more vigorously. For example two latent viruses in mice can be brought to light by this method. Progressive inoculation with lung extracts frequently produces typical pneumonia from which a pneumonia virus of mice can be isolated. Similar serial transmission stimulates into activity Theiler's mouse encephalitis virus.

Little information is found on the effect of the inoculation of body fluids in progressive series through insects though it is probable that a latent virus might easily be activated by this procedure.

Neither is there precise information on the effect of serial passage on latent plant viruses but presumably a tolerant host would continue to be a tolerant host after serial passage through the same plant species. In this connection serial passage of some viruses not necessarily latent through unusual plant hosts produces an apparent change of virulence which usually is due to a selection of one virus strain from a mixture of strains. Possibly during serial passage of a latent plant virus a second latent virus could be picked up on the way when the combination might produce observable symptoms. There are instances in some plants potatoes for example of more than one latent virus existing together.

##### BY THE USE OF ARTIFICIAL IRRITANTS AND CHEMICALS

Many latent viruses can be stirred readily into activity by artificial stimulations of different kinds. A carrier of the Rous I fowl sarcoma reacts violently to injection with methylcholanthrene resulting in the formation of a Rous I

sarcoma. In the Shope papilloma virus in both domestic and cottontail rabbits applications of tar cause the papillomas to become malignant. Here presumably the action is in changing the response of the cell to the virus. Similarly in fowls carrying the virus of fowlpox the virus can be awakened by painting with hydrocarbons. Here again the disease progresses much beyond the normal until a malignant growth develops but identical experiments carried out on fowls free of the virus failed to have any effect.

Andrews points out that the virus of mouse hepatitis apparently exists as a purely latent infection of many stocks of mice. However unlike some latent mouse viruses serial transmission does not stimulate the hepatitis virus into action. The equilibrium can be upset by the injection of another parasite *Eperythron* or coccidia. This appears to activate the virus in some way which becomes evident after further serial passage.

The work of Yamafuji has shown that apparently normal silkworms (*Bombyx mori*) develop polyhedral virus disease after feeding on foliage contaminated with some chemicals such as nitrates. Yamafuji's interpretation of this is that the virus is produced de novo but in view of the very high incidence of latent polyhedral viruses in lepidopterous caterpillars the alternative hypothesis of stimulation of the alternative seems to be preferable.

The lysogenic bacteria represent a different situation from that of most latent viruses. The virus seems not to exist as particles such as occur in vitro for infectious phage cannot be liberated by the mechanical breaking open of the lysogenic bacteria. Its state is indicated by kind of maturation process before it becomes virulent. However it is possible to induce mass lysis and the liberation of infectious phage by certain kinds of artificial stimulation notably ultraviolet light and treatment with hydrogen peroxide.

#### BI INOCULATION WITH A FOREIGN VIRUS

Some good examples of virus stimulation by inoculation with a foreign virus have been found in insects. I understand this it is necessary to mention briefly the different types of insect viruses. First there are the polyhedroses in

which certain tissues become filled with virus containing polyhedral crystals (there are 2 types of polyhedral diseases the nuclear and the cytoplasmic in which the viruses multiply respectively in cell nucleus or cytoplasm) second there are the granuloses in which the tissues become filled with minute granules containing the virus usually a single rod shaped particle per granule and third there are the diseases in which the virus is free in the tissues without intracellular inclusions.

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In higher animals the virus of herpes simplex is frequently stimulated into action by the presence of the common cold virus. When the papilloma virus from the cottontail rabbit is inoculated into domestic rabbits a pap-

loma is produced in which virus cannot be demonstrated. However if the papilloma virus is mixed with the virus of sheep dermatitis and then inoculated to the domestic rabbit papillomas are formed from which active infective papilloma virus can be recovered.

#### BY ENVIRONMENTAL CONDITIONS

Adverse environmental conditions sometimes stimulate a latent virus into obvious activity. For example parrots when kept together under dirty insanitary conditions frequently suffer an outbreak of psittacosis. Also it is common for caterpillars of the puss moth (*Cerura vinula*) to develop a polyhedral virus disease under conditions of high humidity and low temperature.

Environmental conditions such as temperature and light intensity have a great influence on the development of symptoms in plant virus diseases and on the rate of virus multiplication but there does not seem to be any information on the actual stimulation of a latent plant virus by environmental conditions.

#### VIRUS MASKING

Beard considers that masking of symptoms follows simply on a too small virus concentration alone but Shope defines a masked virus as one which is known by circumstantial evidence or by a series of indirect tests to be present but is not of itself directly demonstrable. One example of a masked animal virus is that of rabbit papilloma already briefly referred to. This virus causes warts on the skin of cottontail rabbits in the Middle Western states of the USA and is easily transmitted in series indefinitely in the cottontail. When inoculated into the domestic rabbit similar warts are formed but virus is not transmissible from them either to cottontail or domestic rabbits. However there are indirect means of proving that the papilloma virus is present in the domestic rabbit tumors. These methods are immunologic and make use of the virus neutralization test or the demonstration of active immunity. Luria points out that there is more evidence of the actual existence of virus possibly in modified form in domestic rabbit papillomas. It is occasionally possible to demonstrate the virus in filtrates of extracts of these papillomas by injecting the filtrates into skin rendered hypoplastic by nonspecific irritants. Moreover in 2 cases serial transmission in the

domestic rabbit for 18 and 14 passages has been successful. Return of the virus to the cottontail rabbit for one passage gave rise immediately to a strain of the cottontail type nontransferable to the domestic rabbit.

Reference has been made in the previous section to the experiments of Selbie who was able to obtain active papilloma virus from domestic rabbit tumors if the sheep dermatitis virus was added. We know little of the particular inter-reactions of mixed virus infections and some of the similar phenomena occur in plant virus diseases. Examples are known of 2 viruses occurring together naturally in one plant and while together in the plant both viruses are aphid borne. However when separated 1 of the viruses loses its insect transmissibility.

Shope gives another example of a masked animal virus: this is the virus of swine influenza. Here there is a reservoir host mechanism which keeps the virus going for some 9 months of the year between epidemics. Virus cannot be detected by direct means either in the larval lung worms or in the intermediate host (earthworm) or in the adult longworm after transmission to the pig. Swine which have become parasitized with lungworms known to be carriers of the masked virus do not come down directly with swine influenza. They must have some provocative stimulus and the most successful consists of injections of the bacterium *H. influenzae suis*. Curiously enough this provocation of masked influenza virus succeeds only under experimental conditions between September and April.

Temporary masking of the symptoms in virus diseased plants can be induced by environmental changes. High temperatures are the most effective but low temperature masking is also known. In the case of *Abutilon* mosaic the symptoms disappear when the plants are kept in the dark for a period.

#### MUTATION AND LATENCY IN PLANT VIRUSES

The degree of mutation of which plant viruses are capable is probably much greater than has hitherto been supposed and this has given rise to some interesting problems. The insect transmissibility of a particular plant virus is apparently not an immutable property but is liable to undergo considerable modification in which the host appears to play a part.

Holmes isolated a strain of tobacco mosaic which has changed its relationship in the tobacco plant so that it is completely symptomless in that host. He called this a masked strain but since it is easily transmissible from the tobacco plant it is strictly speaking latent in the sense that observable symptoms are not produced. A similar case was reported by Salaman for potato virus X in the tobacco plant.

By far the most intriguing and puzzling example of plant virus latency is that of potato paracrinkle. This virus was discovered first by Salaman and LePelley and it is latent without exception in every potato plant of the variety King Edward VII. Moreover the virus has no natural means of spread and has never been observed to occur naturally in any other host plant. Therefore the question is how did the virus get into the original King Edward VII seedling in the first place? Only recently has some light been thrown on this problem. Karsan has found that paracrinkle virus is related serologically to 2 other latent viruses: potato virus S and a carnation latent virus. One of the 3 the carnation virus is aphid borne. This suggests a feasible explanation for the paracrinkle virus problem, i.e. that it was carried in the first place by aphids from carnations to the original King Edward VII potato seedling and once there lost by mutation its insect transmissibility. Of course after that the virus would be passed continuously by vegetative propagation in the tubers.

### DISCUSSION

The main questions seem to be: is a latent virus different from a nonlatent virus and is it changed in any way by the various techniques which make it evident? There is no good evidence that a latent virus is chemically or physically different from one which is nonlatent except in one or two possible instances referred to later.

A plant virus which is latent in one host but is virulent in another does not differ so far as we know in any way when extracted from the respective host plants. Most of the differences in severity of symptoms caused could be accounted for by different concentrations of virus and the effect of the various methods of stimulation would be merely one of increasing virus titer. If this is the case it would be more cor-

rect to speak of a "latent infection" rather than of a latent virus.

However we must remember that viruses sometimes can be changed qualitatively by transmission to certain hosts and not only quantitatively. For example we know that one strain of tobacco mosaic virus apparently mutates on transmission between beans and tobacco and potato virus C varies in its insect transmissibility according to its host plant.

However we can differentiate between latency and masking. In the latter state the virus is presumably slightly different from the normal. Although we have discussed the lysogenic bacteria under latency they should perhaps be more correctly dealt with under "masking." Since no phage particles can be demonstrated in the lysogenic bacteria the virus is presumably in a different physical state. Similarly with the Shope papilloma virus and that of swine influenza: in these cases also the viruses seem to be in a noninfectious condition.

We can say tentatively then that we have latency, a condition caused solely by the host virus relationship further we have masking where the virus is actually in a different state. There is the prophage which is presumably an early or resting stage of development and there is a noninfectious stage in some viruses which possibly is caused by a temporary combination of the virus with host-cell elements.

We do not know at present how other incomplete virus forms fit into the picture such as occur with the turnip yellow mosaic and tobacco mosaic viruses and some insect viruses.

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## Viral Inhibition

DR THOMAS FRANCIS JR

In opening the subject of viral inhibition I present broad concepts rather than attempt a detailed review of the manifold studies and the intricate biochemical reactions involved. They have been covered extremely well in recent excellent reviews and symposia. I will try to provide a prospectus as to how viral inhibitors might be introduced to the control of a human virus disease and to consider current indications of the feasibility. The discussion which has been arranged to follow can be expected to amplify or emphasize features of special interest.

Virus inhibition has varied connotations including (a) *in vitro* serologic reactions (b) the normal physiologic influences or barriers and (c) those induced alterations in reactivity which are generally designated chemotherapy or chemoprophylaxis. Study of viral inhibition also is approached variously through investigations at the molecular or cellular level at the more complex level of the organ usually affected or in the intact animal where primary cellular infection, virus distribution beyond the primary site and pathogenomic localization in a more distant vulnerable cell or organ may be progressive stages of infection leading to gross disease in a cellularly complex but integrated host case.

The advantage of having extensive knowledge of the pathogenesis of a disease even though areas of uncertainty do exist as a basis for reasoned approaches to chemoprophylaxis and therapy is well illustrated by poliomyelitis. Studies in man and other primates seem to have established that poliomyelitis is ordinarily an infection of the alimentary tract and that the entry of virus to nerve cells takes place by vascular extension of virus from the alimentary tract. Therefore I shall proceed to the outlook for viral inhibition using poliomyelitis the subject of this Conference as an illustrative text.

In this process one can visualize multiple sites at which viral infection proceeding from benign

cellular involvement to severe disease theoretically may be inhibited by

(1) Preventing the union of virus presumably introduced by mouth with susceptible cells in the alimentary tract

(2) Preventing (a) the penetration and (b) the development of virus in these cells after union has occurred

(3) Eliminating established infection in the alimentary tract

(4) Limiting the multiplication of virus to the alimentary tract so that it does not extend

(5) Intercepting virus in its distribution by vascular systems (blood or lymph) so that it does not arrive in the central nervous system

(6) Limiting or eliminating extension of virus along nerve fibers

(7) Altering nerve cell so as to prevent essential injury even though its peripheral fibers are infected

(8) Inducing changes in nerve cells so as to limit injury and aid recovery

(9) Acting on virus itself after infection is established in the nerve cell

(10) Preventing the extension of virus from infected damaged cells to other susceptible neurones

These are general propositions not implying precise information of the mechanisms involved at the various stages.

What suggestions are there that these stages can be inhibited? What effect could such inhibition have not only on individual cases but also on the epidemiologic aspect of the disease? How could experimentally effective materials be made available to the population?

I. One possibility for preventing the union of virus with susceptible cells of the alimentary tract would be the establishment prior to exposure to the virus of a virucidal activity in the secretions or tissue fluids at the portal of entry similar to the prophylactic use of sulfonamides or penicillin against bacterial infection.

An alternative would be to make available at the portal of entry a substance which not virucidal of itself would prevent the combination of virus with suitable cells. This appears to be the effect of certain amino sulfonic acids which prevent the effective union of influenza virus with susceptible cells but does not destroy the virus: the virus apparently perishes from inhospitability. Recently indications are that the effect of the sulfonic acids results from interference with potassium availability recalling the requirement of certain bacteriophages for tryptophane or inorganic ions to achieve their attachment. Could chelating agents produce a similar effect? The pectin polysaccharides apparently interfere with virus attachment to the cellular substrate. Another possibility is inhibition of a toxic component of the virus which by producing injury at the cell surface may be a significant factor in the efficiency of the virus to combine with cells. The destruction of replaceable cell receptors by RDE is another factor. It is seen then that there are indications that inhibition of cell virus union can be accomplished.

2. Attachment of virus occurs rapidly but does not immediately render virus insusceptible to extracellular influences. Moreover much of the virus which combines with the cell may fail to gain entrance. Thus substances which would delay or further reduce incorporation of virus with the cell could be sought. They could result either in virus disintegration or in rendering it more readily disposable by the body. Virus interference may have such an effect. Here enhancement of normal physiologic inhibitors may be of importance since they tend to increase spontaneously with injury. It seems likely that inhibition of viral progress at this introductory stage is achieved commonly by natural defense mechanisms in view of the high proportion of exposed subjects who escape without evidence of effective contact. However Bawden has expressed the opinion that most inhibitors of plant virus infection act more on the cell to delay entry of virus or to stimulate cells to an unusual activity which renders virus noninfective. He mentions that leaves treated with trichothecins after presumed infection are refractory but regain susceptibility in about 2 days which roughly resembles the effect of RDE on cellular receptors. This stage of infection that is primary cellular penetration is under usual condi-

tions an unrecognized period except during epidemics or situations of well identified exposure as with measles, smallpox or rabies. Therefore ordinarily an effect on this early phase almost of necessity would have to be pre-established and induced alterations in the cells would have to be physiologically endurable for considerable periods of time. In that respect the first two steps almost may be looked at as the same problem. The outlook is essentially the prevention of infection.

Prevention of the development of virus which has become vitally established in the cells is the focus of much attention. Tissue cultures are especially suitable to this type of investigation since they permit close observation of growth curves under relatively well-defined conditions. Great numbers of materials have been tested: antimetabolites of many kinds, metabolic analogues, dyes, energy and oxidation inhibitors, antibiotics, polysaccharides and synthetic polypeptides. Examples of nearly all of these classes have been shown to exert an effect in limiting infection and the yield of assorted viruses. Metabolic analogues are of special interest since they permit identification of specific effects by their specific reversion by the normal compounds. Their use has indicated the practicality of metabolically blocking the formation of virus under physiologically tolerated conditions. They also permit designation of distinct steps in virus development and demonstrate the susceptibility of the different steps to different influences. They have one disadvantage in that resistance to the metabolite commonly develops. The studies with methoxymine and fluorophenylalanine have been among the most informative concerning animal viruses; those with benzimidazole derivatives have been among the most consistently progressive in designing compounds of increasing effectiveness. Although there has not yet emerged a clear design for integrated interpretation of the numerous observations, they emphasize repeatedly that intracellular virus infection can be influenced to the advantage of the host by induced alterations in the metabolic processes of the cell. One might interpret that this may be accomplished much more readily in a specific basic type of cell like the lymphocyte. The results also point out that the cell is not merely a subservient biochemical community irrevocably at the whim of a dictatorial virus.

that the cell is not entirely a passive participant in virus union penetration and development.

The possibility has been expressed that cells disturbed by virus infection may be detected selectively by chemical compounds and destroyed so that developing virus also succumbs. If this occurred to a limited extent in the superficial or lymphatic cells of the alimentary tract bearing the initial infection theoretically no major harm would be done. However if anterior motor neurons showing evidence of injury were destroyed an exaggerated paralytic disability would ensue.

This like the 2 preceding steps represents prevention of virus establishment preparation for which would be required in advance. How could this be accomplished practically? One readily can visualize the administration of an effective chemical to the general population by distributing it throughout the poliomyelitis season in some simple vehicle like iodine in salt fluoride in water or reinforcements in bread or milk.

3 Most biochemical studies have been directed on un-co-ordinated virus-cell systems resulting in disruption of the cell. However virus infection is commonly of such a nature that the virus does become sufficiently co-ordinated with the synthetic activities of the cell to create an integrated cell virus relationship which can persist.

To some extent this appears to be the situation encountered in continued infection of the alimentary tract where Podians data indicate that the lymphoid tissue is heavily infected with out obvious anatomic alterations. The ability to eliminate that infection it will could be a valuable asset epidemiologically in reducing opportunity for transmission and clinically by reducing the probability of extension of the virus to the CNS so as to produce paralytic disease. The studies of Ward *et al* have suggested that certain mercurials can be effective in reducing alimentary virus infection. However note that Koprowski and his associates were unable by administration of calomel to eradicate the alimentary virus infection being maintained in human subjects after feeding Type I polio virus. Nevertheless observations with other compounds such as betapropiolactone or certain detergents warrant further exploration into the destruction of alimentary-excreted virus. This may be of great value not only re polio virus

but also re ECHO and Coxsackie agents. But inevitably treatment of a well balanced infection of this nature will require particular consideration of location of the infection including the character and the function of the cell and the tissue involved. However if poliomyelitis is primarily a virus infection of the lymphatic cells the specificity should increase the probability of discovering selective inhibitors of continuing virus development. However prompt elimination may necessitate a multiple approach which will affect cell permeability produce a rapid metabolic imbalance and inactivation of the exuding virus. Cortisone might be suitable as a disturber of the truce. Specific antibody may well be used as an adjunct in this objective although the persistence of alimentary infection strongly suggests that it is in an extravascular location.

4-5 The next approach is that if alimentary infection is not eradicated it should be limited sharply. There are numerous recommendations for the idea of intercepting virus being released by cells of the alimentary tract which may by vascular dissemination reach the important motor neurons. Much of this virus is extracellular and not yet in position to combine immediately with the specific vulnerable cells. Therefore it should be more subject to combination with inhibitors or inactivators. There are strong indications that it is true. The influence of antibiotics such as helmin and 8450 on MM and Semliki Forest viruses in mice suggests that it is a viremic stage or a stage leading to viremia which is interrupted after peripheral introduction of virus. Both these materials given parenterally have a protective effect on monkeys infected with poliomyelitis virus either subcutaneously or by feeding. There is some evidence that the production of virus in the alimentary tract is reduced but the action is not a direct action on virus in the alimentary tract as shown by the fact that giving the antibiotics by mouth has no effect on development of paralysis. Therefore it must limit the dispersion of virus in the body. Monofluoroacetate is a compound which was shown to reduce the extent of viremia in monkeys while providing protection against paralytic disease. The effects observed with Atabrine are most distinct with the viremic neurotropic infections of mice induced intramuscularly. Presumably some of the reported



effects with dyes may be exerted on virus in the blood stream

With many other compounds the results noted either positive or negative with different viruses and routes of virus administration contain too many variables to permit a significant impression as to site of effect. Other materials under study in our laboratory give promise of significant protective effect against poliomyelitis in monkeys again with influence directed against circulating virus

Matthews has demonstrated that the guanine analogue 8 azaguanine when sprayed on leaves as late as 2 days after infection will prevent systemic development of certain plant viruses but it or other compounds were without effect after systemic involvement had begun. He also noted that the analogue was incorporated into the virus formed early in the infection with a marked reduction in its infectious capacity. This suggests that the influence of metabolic inhibitors is not necessarily limited to the cell only. As suggested earlier it seems likely that vaccination with inactive virus exerts its major influence against paralytic poliomyelitis by neutralizing virus which may be circulated by the antibody developed. It does not prevent the elementary infection but protects the nervous system. The effect of vaccine strongly supports the outlook for chemical inhibition of poliomyelitis virus in the vascular systems

6-10 The next possible sites of viral inhibition considered are in many respects reconsideration of the steps involved at the portal of entry except that they now refer to a second stage in pathogenesis in which specific nerve cells are the target. Stepwise these are advanced stages of virus infection but may be considered to be preliminary stages of paralytic poliomyelitis. Where and how do the nerve fibers and the vulnerable nerve cells become infected? Bodian suggests that it may naturally occur by overflow of virus into the area postrema. Certainly there are strong indications that under certain conditions—tonsillectomy operation overactivity inoculation—the virus may proceed directly along fibers toward the neurone. The effect of sectioning the nerve injections of alcohol cocaine or other materials which alter its physiologic state may reduce extension along the nerve. Thus substances which have a direct inhibitory or mimetic influence on nerve physi-

ology are of interest or substances acting through the main cell body may be influential.

The stages discussed earlier can be presumed to be prophylactic in terms of paralytic disease although therapeutic of the infection. Any procedure which can reduce quantitatively the number of neurones affected or the severity of injury to the neurones may be of importance in therapy of paralytic poliomyelitis. One is faced with ignorance of the mode of entrance of the virus into the nerve cell or of the biochemical mechanisms of the injury. The destroyed cells cannot be expected to regenerate. But from Bodian's observations 80 per cent of the neurones which histologically appear to be affected may recover and this points to the possibility that chemotherapy may be able to speed that restoration. In fact the question is whether much of that injury is a toxic effect as has been demonstrated by Ackermann, Payne and Kurtz in tissue culture rather than a result of free virus multiplication. In this event substances like Verosin which reduce toxic effect of viruses could be useful. Or are these restorable neurones ones whose infection has been interrupted in the peripheral processes?

If knowledge of the substrate with which poliomyelitis virus combines in the cell can be obtained it may be possible to prepare a protective inhibitory analogue. This possibility is implicit in our demonstration of a protein in the gray matter presumably in the neurones of the CNS which combines selectively with poliomyelitis virus. The studies of Hyden of the effect of malononitrile on the nucleoprotein of the motor neurones led to examination of that compound's influence on experimental disease. Although at times some influence was noted malononitrile gave no significant protection. Finally if a limited number of cells is initially infected the introduction of inhibitors which prevent further development of virus or attachment to new cells could at this stage be beneficial.

This broad view of approaches to viral inhibition in terms of the mechanisms of pathogenesis of a virus disease has served primarily to emphasize that the outlook does not have to be limited to one theme or one stage of infection. The point of view also may shift according to whether the intent is prevention or treatment. Clinically the term preventive or prophylactic can be

applied to any effect attained prior to onset of manifest disease and therapy only when disease has become apparent. Experimentally when the time of infection is known it is our tendency to call prophylaxis any effect obtained in animals by administration of the test material before infection and therapy effects gained after infection. In tissue-culture systems this same experimental designation applies.

At this point I make a few remarks about the test systems. The use of tissue or cell cultures has obvious advantages. They have permitted much learning of the different stages in viral development and how they can be inhibited. But this information has not ordinarily sustained its promise when tested in the complex medium of the susceptible animal. That failure points to the difficulty of attaining in one the conditions readily encountered in the other. In cultures the cells or tissues are separated from the controlling influences of the total organism; they are likely to be of a type quite different from the highly differentiated cells of the body characteristically damaged by the virus under study. The system has probably become adjusted so that virus readily encounters suitable cells and an abnormally brief cycle of virus development occurs so that virus might be more difficult of inhibition. The virus may be changed so that it is no longer like the natural prototype. Consequently greater attention to these factors might lead to better translation of effects to the whole animal.

### CONCLUSION

The best composite view of viral inhibition is that presented by the naturally resistant species. This state seems strongly to recommend greater effort to understand the conditions which govern that inhibitory action of the nature of varied serologic influences, natural antibody information of the behavior of cells in animals with solid or partial natural immunity vis à vis the susceptible. These need study for understanding of cell-virus relationships. I emphasize once more a thesis that I have presented repeatedly—that in study of viral inhibitors we must pay more attention to identification of the virus action and its specific substrate; we must recognize that the cell is not a passive fixture in this reaction and that fixation and penetration of virus may be more the active operation of the

cell than of the virus. Finally in contrast with the outlook commonly followed for cancer chemotherapy our purpose is not to destroy these highly necessary cells but to support and restore them.

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## DISCUSSION

DR DELBRUCK Poliovirus allow the study of the genetic properties of a biologic system having ribonucleic acid as the only or main nucleic acid. This study is particularly interesting since all the systems the genetics of which is fairly well understood contain deoxy ribonucleic acid. A model for the latter systems are the bacteriophages - the genetics of which has been discussed today by Delbruck.

Our laboratory has been engaged in the study of poliovirus genetics for some time and a number of results have been secured. The work that I am going to discuss briefly concerns essentially a study of the mutability of polio virus both spontaneous and induced.

### SPONTANEOUS MUTABILITY

The spontaneously occurring mutants which were isolated and studied in our laboratory are

- 1 Heat resistant mutants. They differ from wild type virus for their smaller rate of inactivation under identical conditions. Different types of these mutants occur having different inactivation rates. A recent finding may lead to an understanding of the structural changes produced by the mutations. In fact in collaboration with P. Pohjanpelto we have recently discovered that the sensitive virus can be made as resistant as the most resistant mutant by exposure to cystine.

- 2 Mutants having a reduced efficiency of plating under an acid overlay. They are called *d* mutants where *d* stands for delayed plaque appearance. The wild type virus which has a high efficiency of plating under acid agar is called *d*<sup>+</sup>. These mutants have a special interest because they are frequently attenuated for the nervous system of the monkey although with some exceptions.

- 3 Mutants having different plaque sizes similar to those described by Sabin. The interaction between these mutants was studied. They occur independently since most of the possible combinations were obtained by successive mutations. However there are some distinct interactions. Thus it was impossible to isolate a temperature resistant *d* mutant whereas temperature resistant

*d*<sup>+</sup> mutants can be isolated very easily. Perhaps the former combination is lethal or the expression of one of the characters is suppressed by the other. Another example of interaction is the decreased *d* character of large plaque mutants of *d* virus.

The *d* mutants give rise to reversions in the *d*<sup>+</sup> direction. A quantitative study of the *d* to *d*<sup>+</sup> mutations can be carried out since by plating on acid agar the reverted virus particles can be detected and counted when they occur in a proportion of 1 in 1 000 000 of *d* particles. By using this system it was possible to prove by standard microbiologic techniques (the fluctuation test of Luria and Delbruck) that the *d*<sup>+</sup> reversions occur spontaneously at any time during the growth of the *d* virus. The mutation frequency of certain mutational steps could also be measured by the same technique and was found to be of the order of 1 in 100 000 *d* particles per viral duplication.

The expression of the *d* character can be measured in a quantitative way. Such a measure is given by the ratio of the virus titer on acid and alkaline plates respectively and is defined as the efficiency of plating acid. The efficiency of plating acid can vary between 1/1 000 or 1/10 000 for high-efficiency *d* mutants and 1/1 000 000 for low-efficiency mutants. The reversions obtained from high-efficiency and low-efficiency mutants (respectively) have different properties as will be shown presently. High efficiency mutants revert apparently in 1 step to virus which is very similar to wild type in *d* character. In collaboration with H. A. Wenner we could prove that the reverted virus is also similar to wild type in neuropathogenicity. Low efficiency *d* mutants revert to types intermediate in *d* character. By continued selection several intermediate types of increasing *d*<sup>+</sup> phenotype can be isolated however a real *d*<sup>+</sup> virus is never obtained. In collaboration with A. B. Sabin we could show that these reversions maintain the character of attenuation.

Due to the extensive correlation between *d* character and neuropathogenicity it is likely that in these cases the *d* mutants are due to

genetic changes affecting loci which control both characters simultaneously. The following picture may be suggested.

The high efficiency of mutants are more complex since they do not give rise to complete reversions with a measurable frequency. This fact and the more pronounced phenotypic effect that they display both in relation to  $\delta$  character and neuropathogenicity suggest that they may be deletions at a  $\delta$  locus. The reversions for the  $\delta$  character of these mutants can be attributed to mutations at suppressor loci which affect only some of the phenotypic effects of the deletion.

Now consider the problem of the induction of mutations in polio virus by mutagenic agents. Experiments on mutagenesis could be carried out by using the mutations from  $\delta$  to  $\delta^+$  of a high-efficiency  $\delta$  mutant. Three agents so far were investigated: x-rays, ultraviolet light and proflavine. Either radiation was used to irradiate virus infected cells rather than free virus as in previous work with bacteriophages had shown that this was the most suitable way for inducing viral mutations. Although a large variety of doses of radiation was used and the irradiation was given at various times during the latent period, no mutagenic effect was observed in either case. Proflavine on the contrary was found to be effective. Infected cells were exposed to different concentrations of the drug during the whole period of virus multiplication (about 74 hours). At adequate concentrations of proflavine the total virus yield from these cells was depressed and the proportion of mutants in the yield was increased, the 2 effects being correlated. The maximum effect obtained was a nearly tenfold increase in the proportion of mutants over background. A selective effect of proflavine on the mutants was excluded. We do not know as yet whether proflavine is effective in increasing the proportion of other mutations as well. However, since mutagenic effects are generally non-specific it may be suggested that a proflavine treatment should precede any attempt to isolate mutants of poliomyelitis virus ended with special genetic properties.

We add the following concluding remarks. One of the facts that begins to emerge from the reported studies and others which were not discussed here is that the genetic and reproductive properties of poliomyelitis viruses do not

differ fundamentally from those of some DNA viruses such as the even numbered bacteriophages of *E. coli*. It is particularly striking that the same agents are mutagenic in both cases. The observed similarities suggest that although the 2 types of viruses have a different nucleic acid they may have some very similar step in their multiplication.

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Dr S HAYER. In his impressive review Dr Horsfall pointed out that ribonucleic acid (RNA) is sufficient to cause the formation of new virus particles not only in the tobacco mosaic virus but also in the Mengo encephalitis virus.

In addition an infectious principle having properties similar to the material obtained with the Mengo virus was isolated recently at our Institute from the United States strain of equine encephalomyelitis (Eastern type).<sup>1</sup> According to experiments carried out thus far it can induce the formation of new infectious particles both in the brain of mice and in the allantois of chick embryos. However it is quite distinct from the usual infectious agents on account of its sensitivity with respect to ribonuclease its lability in the presence of high temperatures and its behavior when precipitated with alcohol (see Table 176). These are either partly de-

TABLE 176. BEHAVIOR OF THE ENCEPHALOMYELITIS VIRUS AND OF THE ISOLATED RNA FRACTION

TREATMENT	VIRUS PREPARATION	RNA FRACTION
	MLD <sub>50</sub> per cu cm (-log)	MLD <sub>50</sub> per cu cm (-log)
Initial value	8.1	3.0
Ribonuclease	7	0
4 hours at 37°C	6.7	0
Net of precipitated	0	3.5
MLD <sub>50</sub> of chick embryos		
1000 times dilution		

1. Error is indicated with standard deviation.

composed virus particles which the RNA has released or the RNA of the virus particle itself. Further experiments to determine the characteristics of this material are now in progress.

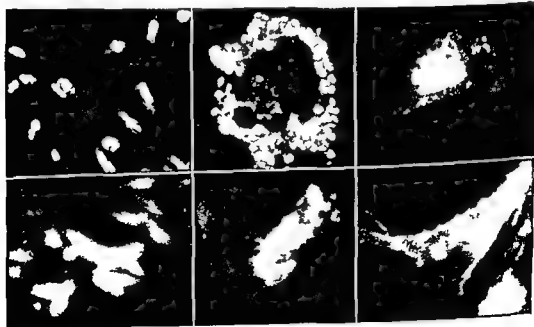
Our efforts to isolate a pertinent component in the virus of classic fowl plague—which has been investigated more intensively by us in the last few years and nucleic acid which as in the case of the virus of equine encephalomyelitis is present only in the form of ribonucleic acid—have thus far persistently failed. This may

be due to the fact that the experimenter could not reproduce the invading mechanism of the structurally more complex virus of fowl plague.

However, it has been possible to obtain by other means a considerably more complete idea of the course and the nature of the reproduction of this virus.

In order to present these results I shall discuss briefly the structure of the virus particle of classic fowl plague. As shown in the diagram at the top of Figure 227 it consists of at least

FIG. 227 (Top) Diagram of the virus of classic fowl plague (Center left) Embryonal chick cells 3 hours after infection. Fluorescence of the nuclei after treatment with fluorescent II antigen antibody (Center middle) Chicken macrophage giant cell  $3\frac{1}{2}$  hours after infection. Fluorescence of the nuclei after treatment with fluorescent II antigen antibody (Center right) Embryonal chick cell 10 hours after infection. Fluorescence of the nucleus and of the cytoplasm after treatment with fluorescent B antigen antibody (Bottom left) Embryonal chick cells 4 hours after infection. Fluorescence of the entire cell with greater fluorescence at juxtannuclear points. Fluorescent hemagglutinin antibody (Bottom middle) Chicken macrophage giant cell 4 hours after infection. Fluorescence at the cytoplasm. Nuclei blank. Fluorescent hemagglutinin antibody (Bottom right) Embryonal chick cell 14 hours after infection. Greater fluorescence at the cell periphery. Fluorescent protrusions. Fluorescent hemagglutinin antibody.



2 types of subunits of which the internal bound or B antigen is the bearer of the RNA of the virus particle whereas the hemagglutinin which lies on its surface does not contain any nucleic acid but does possess the receptor-destroying enzyme and the adhering groups of the virus.<sup>19</sup> In regard to all the properties tested the S antigen encountered outside the virus particle corresponds to the B antigen.<sup>8</sup>

It could be shown with the aid of virus particles whose B antigen was labeled by incorporation of  $P^{32}$  into its RNA that this virus too—just as the influenza virus<sup>6</sup>—disintegrates when the host cell is infected releasing its B antigen and from this probably even the PNA.<sup>31</sup>

The resulting formation of new virus material in the cell was recently investigated by means of fluorescent antibodies<sup>5</sup> in infected tissue cultures.<sup>14</sup> The impression was gained that the B antigen is formed in the nucleus. It can be demonstrated there first 2 to 3 hours after the infection (Fig. 227 center left and middle). Later it is also observed in the cytoplasm (Fig. 227 center right). The hemagglutinin which appears an hour later than the B antigen is present right away throughout the cytoplasm (Fig. 227 bottom left and middle) and is encountered in higher concentrations in a juxta nuclear position the significance of which is not yet clear to us at this time. It later accumulates along the periphery of the cell where it also can be demonstrated in the form of fine protrusions (Fig. 227 bottom right). We know from ultrahistological investigations that it is only here that the new virus particles are formed.

The observations described indicate that the two known components of the classic fowl plague virus are formed not only at different times but also at different places in the host cell.

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DR. TASAKI: The manifold aspects of the process of virus multiplication and the various approaches to virus inhibition have been set forth comprehensively by earlier speakers. I confine my remarks to approaches to selective inhibition of virus multiplication through chemical interference with the biosynthesis of nucleic acids or proteins.

It is generally agreed that the process of virus multiplication is closely linked to the metabolic activities of host cells. The fact that synthesis of virus materials inside susceptible host cells takes place in the absence of a limiting virus membrane reflects the intimacy of the host virus relationship. This situation is understood on the fact that all of the chemical compo-

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have been used to inhibit virus multiplication have been found to have some effects on host cell metabolism at virus inhibitory concentrations wherever such effects have been looked for. In spite of this certain compounds are sufficiently active and selective to reduce the yield of virus in intact animals and to protect them against death from virus infection under restricted experimental conditions.

Both the compound and the virus employed are important determinants in experiments on inhibition of virus multiplication. Depending on the chemical structure and biochemical mechanism of action different chemical compounds interfere preferentially with different metabolic reactions. Furthermore definite differences exist among virus inhibitory compounds with respect to their activity and toxicity.

In studies on virus inhibition with benzimidazole derivatives we have found that virus inhibitory activity and toxicity of compounds may vary independently; certain modifications in the structure of the inhibitor molecule have resulted in a disproportionate increase in influenza virus inhibitory activity relative to toxicity. Among such derivatives showing enhanced selectivity are the  $\beta$  linked D ribofuranosides of halogenated benzimidazoles. These compounds are among the few which are sufficiently active and selective to cause appreciable inhibition of influenza virus multiplication in mice and chicken embryos. These ribosides of halogenated benzimidazoles are new inhibitors of ribonucleic acid biosynthesis and they appear to act as antagonists of a natural purine riboside. Any departure from the ribose structure in the pentose moiety results in reduced influenza virus inhibitory activity and selectivity.

The nucleic acid in influenza virus particles is of the ribonucleic acid type. With the aid of the riboside of dichlorobenzimidazole it has been shown that virus nucleic acid synthesis precedes the formation of the more complex viral structures such as the soluble complement fixing antigen and virus particles.

The nucleic acid in poliomyelitis virus is also of the ribonucleic acid type and as was expected the ribosides of halogenated benzimidazoles proved to be active as inhibitors of poliomyelitis virus in monkey kidney cells *in vitro*. Studies with poliomyelitis virus were carried out in collaboration with Dr. Marjorie M.

Nemes. It should be emphasized that the ribosides were less active against poliomyelitis virus than against influenza virus. Since both poliomyelitis and influenza viruses are ribonucleic acid viruses and since the ribosides of halogenated benzimidazoles reduce the rate of synthesis of ribonucleic acid an explanation for this difference in activity must be sought in the circumstances which surround the synthesis of virus materials inside host cells.

At the higher concentrations required to inhibit poliomyelitis virus the ribosides were toxic to cells. Unexpectedly another glycoside of dichlorobenzimidazole, namely the  $\alpha$  linked D-arabinopyranose derivative did show enhanced selectivity against poliomyelitis virus. To elucidate the biochemical basis for the difference in selective action we have studied the effects of the riboside and the arabinoside on ribonucleic acid and protein synthesis in monkey kidney cells. Although both compounds inhibited ribonucleic acid synthesis at concentrations inhibitory for the poliomyelitis virus the arabinoside which is more selective for this virus also had a marked inhibitory effect on protein synthesis. Furthermore compared with the riboside the arabinoside could be given later after inoculation of virus and inhibition still obtained. In fact some effect was obtained even after logarithmic increase in virus had begun.

These observations suggested that inhibition of protein synthesis provided an approach to the selective inhibition of poliomyelitis virus multiplication which was preferable to inhibition of ribonucleic acid synthesis. This tentative conclusion has received additional support from results of more recent experiments with 2 entirely different compounds, azaserine, an inhibitor of ribonucleotide synthesis and  $\beta$ -fluorophenylalanine, an inhibitor of protein synthesis.

The available inhibitors of protein synthesis in mammalian cells are all compounds of rather low activity on a weight basis. For this reason alone they do not offer promise of effectiveness in intact animals. It is to be hoped that through discovery or development new and more active inhibitors of protein synthesis will become available for studies on virus multiplication and other important biologic processes.

Dr. Zinder. Dr. Delbruck has told of some of the wonderful and extraordinary things that

occurred during the vegetative growth of bacterial viruses. There are two things that a bacterial virus (bacteriophage) may do to a bacterium other than destroy it. For this we need the class of bacteriophage called "temperate" which do not destroy all of the infected organisms but rather in some fixed proportion dependent on the host the nature of the bacteriophage and the environment induce a lysogenic or carrier state.

I digress for a few moments with regard to the phenomenon of lysogeny. Following infection with a temperate bacteriophage almost all of the organisms survive and continue to grow but release during growth with a fixed frequency per generation a bacteriophage. Therefore these bacteria carry the potentiality to produce bacteriophage at some later time. This potentiality has been called prophage. In general it is now known that a cell can carry only a single prophage of a particular kind and in all probability this prophage is carried on or in the bacterial chromosome. The most important evidence of this comes from studies on *E. coli* where in crosses between lysogenic and nonlysogenic bacteria prophage segregates as to the other bacterial markers and in fact turns out to be linked to other bacterial markers. The lysogenic bacterium exhibits the property of being immune to superinfection by the identical phage which it is carrying. This then is one property of a phage-infected bacterium not of the kind of which I shall speak today. We need such a system in order that the bacteria should survive the infection.

I restrict this discussion to one such system—the one that I have been working with—and our host will be the mouse pathogen *Salmonella typhimurium* and a temperate bacteriophage which we have called P22. For the sake of simplicity and clarity we will reverse the terminology in the study of these phenomena.

The first phenomenon has been called lysogenic conversion that is lysogenized cells exhibit a new property which is not obviously related to the presence of the prophage. The most striking example which was discovered in modern times is that of the diphtheria organism which produces the diphtheria toxin only when it is lysogenic and does not produce it when it is nonlysogenic although again deferring to our human historical sense probably there were

reports of similar phenomena in the 1970's when it was reported that the streptococci producing scarlet fever were also lysogenic and the erythrogenic toxin probably was controlled by the presence of bacteriophage.

In our system—the *Salmonella typhimurium* system—when the cell becomes lysogenic for the bacteriophage P22 it is not only immune to secondary infection but also exhibits a new somatic antigen. This somatic antigen is the somatic antigen I which long has been known in the bacteriology of *Salmonellae* and has been used as a diagnostic criterion of the antigenically complex *Salmonellae*. About 10 minutes following infection with the bacteriophage the antigen is sufficiently developed to be detected by the rather crude serologic means of cell agglutination. When lysogenic bacteria are cured of the prophage as they can be by appropriate ultraviolet treatment the cells eventually will lose this antigen. Thus every lysogenic cell exhibits this property and loses it following loss of the phage.

The other phenomenon mediated by bacteriophage is what we have called transduction. Herein following infection by a temperate bacteriophage a small proportion of the treated bacteria is altered in such a way as to be a reflection of the genetic constitution of the host upon which the phage last grew. Let me cite an example.

Given a donor bacterium which is capable of fermenting the sugars galactose and xylose the phage is grown on such a strain. Given a recipient which is incapable of fermenting either sugar either as obtained in nature or more readily obtained in the laboratory by sequential mutational events we then infect this recipient and among the progeny of these infected cells we find organisms which are capable of fermenting either sugar but not both. They occurred with the approximate frequency of 1 in 1,000,000 per phage particle. In the *Salmonellae* with this particular bacteriophage almost all bacterial properties can be transduced. These include such properties as drug resistance nutritional requirements and flagellar antigens which do not seem to be under the direct control of lysogenic conversion. This phenomenon of transduction is distinguished from general mating by the fact that only a part of the bacterial genome is transferred in any particular event.



### RATE OF DEVELOPMENT OF CYTOPATHOLOGY

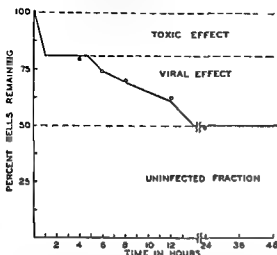


FIG 228 Data are a composite of two experiments. Each point represents a single experimental culture. Per cent cells remaining =  $100 \times \text{cell count of experimental culture} / \text{cell count of control culture}$ . Each experimental culture was exposed to 7.4 PFU of virus/cell for 15 minutes.

### DOSE RESPONSE OF TOXIC FLUID

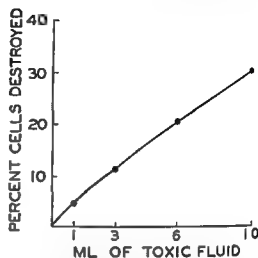


FIG 229 Replicate cultures of cells were exposed to 4 ml of balanced salt solution containing amounts of toxic fluid ranging from 0.1 to 10 ml. After exposure for 30 minutes cultures were washed 5 times to remove the infecting fluid and incubated at 37° in maintenance solution for 45 minutes. At the end of this period the surviving cells were determined. The per cent of cells destroyed was determined by comparison with a control culture.

Therefore we see that in addition to cytopathogenic effects of viruses at least some bacterial viruses are capable of producing 2 types of permanent modification and therefore probably genetic modification of their hosts. In one the property seems to be controlled directly by the presence of the phage genome—this being the lysogenic conversion for loss of the prophage involves the loss of the property. On the other hand in transduction it is probable that the virus can be viewed only as a passive vector of fragments of genetic material, a kind of casual contamination of the virus. I do not know of the existence of such phenomena in animal or plant viruses but so many of the techniques that are used in bacteria are dependent on the ability to maintain pure clones of organisms and high use of selective procedures that the developments described here yesterday portend the discovery of similar and further phenomena in animal and plant viruses.

DR ACKERMANN: It is quite likely that when the useful application of viral chemotherapy is realized the essence of the principle involved will

be found to have been considered in one of the 4 papers presented this morning. At present it remains for some shrewdly discerning individual among us to recognize by foresight which principle is the essential one.

For myself I was impressed with the diverse cell responses which Dr Smith described for the varied host-virus interactions. It is this type of observation of comparative virology which led us to question whether or not in a single host-virus system viral multiplication and cell destruction result from autonomous reactions. Indeed it has been possible to show that substances such as p-fluorophenylalanine which in tissue culture completely prevents increase of polio virus do not prevent infection nor alter the subsequent typical viral cytopathology. A recent observation in our laboratory is pertinent in relation to this observed degree of autonomy

of the cytopathogenic effect produced in HeLa cells by infection with polio virus

A toxic effect of fluids obtained from tissue cultures infected with Type 1 polio virus was observed when the fluids were introduced into fresh cultures. The destructive activity is distinct from the usual virus induced cytopathogenic effect in that it occurs rapidly without a prolonged latent period and without virus production. This is illustrated in Figure 279 where the cytopathogenic activity of an infected tissue culture fluid on HeLa cells was followed quantitatively as a function of time. The data show that a loss of cells from the glass substrate of the culture occurs within 2 intervals 1 within the first hour and the second between the sixth and twelfth hours. Between the first and fourth hours and in the period from 24 to 48 hours the cell count was constant. Under these conditions intracellular virus does not appear until sometime between the third and fourth hour after infection. There is a distinct intracellular phase and release of virus from the cells begins between the sixth and seventh hour.

The response of cultures to various concentrations of tissue-culture fluid was determined. In Figure 279 the percentage of cells destroyed is plotted as a function of the concentration of the infected fluid present in the exposure period. It is seen that at low concentrations of the range studied the response of cells was nearly linear while at the higher concentrations the response appeared to be diminishing. The data strongly

indicate that the rapid cell destruction is the result of a specific material in the fluid. Dilutions greater than tenfold do not produce effects which are measurable with a curacy.

The infectious activity can be neutralized selectively by certain antiserum with full retention of the toxicity i.e. with antiserum prepared by Wenner in monkeys using infected monkey cord material. However antisera prepared with infected tissue-culture fluid also will neutralize the toxic effect. The antitoxic effect of such sera may be selectively adsorbed with hophilized preparations of HeLa cells but antibodies prepared against uninfected HeLa cells do not show an antitoxic effect further the two activities possess differential heat stability.

It should be emphasized that the ability to destroy cells and give rise to detectable amounts of toxin is a characteristic of only certain lines of the Mahoney strain of Type 1 polio virus. In a comparative study 3 lines were investigated these are designated PT and 11. These lines are also distinct in their plaque morphology. The PT line produces large rapidly forming plaques and gives rise to toxic tissue-culture fluids. The 11 line is characterized by small late forming plaques and non-toxic fluids.

It is interesting to speculate that the toxic activity of the tissue-culture fluid observed in fresh cultures is the same which produced the cytopathogenic effect and accompanied virus production in the culture of its origin.



# Diagnosis

FRIDAY MORNING, JULY 12, 1957

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## *Chairman*

DR WILSON SMITH  
Department of Bacteriology  
University College Hospital Medical School  
London

## *Speakers*

DR EDWIN H LENNETTE

Viral and Rickettsial Disease Laboratory  
California State Department of  
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DR ALBERT B SABIN

Children's Hospital Research Foundation  
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St Vincent's Hospital  
Department of Pathology  
Dublin

PROF F PIETSCHYCKI

Institute of Hygiene  
Warsaw



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# Diagnosis

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# Problems of the Viral Diagnostic Laboratory with Respect to Poliomyelitis

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Application of the newer tissue-culture techniques to the laboratory diagnosis of poliomyelitis has not only greatly simplified the diagnostic methodology but also has made it possible to apply diagnostic tests on a scale impossible only a few years ago. However procedural simplicity is not necessarily commensurate with diagnostic infallibility and each of the several tests currently in use has its own shortcomings. Throughout this discussion I shall speak primarily from the viewpoint of a viral diagnostic laboratory and not from that of a viral research laboratory. I mention this since it seems likely that in some respects the experience of research laboratories may not coincide entirely with ours. I should like then to say something of the problems and the difficulties encountered by a laboratory which undertakes to assist the clinician in establishing or ruling out a diagnosis of poliomyelitis by virus isolation attempts and/or serum antibody assays.

## DIAGNOSIS BY ISOLATION OF VIRUS

Virus isolation is regarded as a more useful diagnostic test than either of the serologic meth-

ods since it affords a means of making an early diagnosis. It is generally considered that through the use of tissue-culture techniques the virus can be recovered from approximately 90 per cent of early paralytic cases or serologically confirmed nonparalytic cases if the stool specimens are collected properly and during the early stages of the infection. The key to the successful attainment of such a high viral recovery rate would appear to reside in the words "properly collected stool specimens and early in the illness." On the whole these stipulations can be carried out fairly well when the collection of clinical specimens is under the control of the investigator or of well indoctrinated collaborators. However even such control does not necessarily lead at least in our experience to viral recovery rates greater than 75 to 80 per cent.

The basis for requesting and examining multiple stool specimens from patients with suspected poliomyelitis is illustrated in Table 127. The results of virus isolation studies on serial stool specimens indicate (Table 128) that shedding of the virus in the feces is not necessarily a continuous process but apparently a discon-

TABLE 127 RELATION BETWEEN INTERVALS PROPOSED FOR COLLECTION OF STOOL SPECIMENS AND INTERVALS ACTUALLY FOLLOWED BY PHYSICIANS SUBMITTING SPECIMENS

SPECIMEN	STOOL SPECIMENS REQUESTED		TOTAL NUMBER SPECIMENS	INTERVAL STOOL SPECIMENS COLLECTED		
				MEAN	MEDIAN	RANGE
	Prior to June 15 1955	After June 15 1955				
1st	Soon as possible after onset	When patient seen and 3 to 4 specimens taken at 3 to 4 day intervals during the early phase of the illness	87	11 days	9 days	0-53 days
2nd	3 days postonset		758	17 days	15 days	2-73 days
3rd	6 days postonset		573	27 days	21 days	3-73 days
4th	10 days postonset		378	77 days	27 days	5-73 days
5th	Not requested		113	27 days	27 days	13-43 days
6th	Not requested		55	30 days	30 days	21-44 days
7th	Not requested		38	31 days	35 days	27-51 days



TABLE 128 DISCONTINUOUS NATURE OF VIRUS EXCRETION IN POLIOMYELITIC PATIENTS

NAME	AGE	SEX	CLINICAL STATUS	VIRUS IN STOOL TYPE	DAYS AFTER ONSET OF ILLNESS																		
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	19	20	21
W M	11	M	Paralytic	1	+					+	+	-											
E C	8	F		1		+		-	+	-	-												
E D	32	F		1		+		-	-	-													
R K	1	M		1			+	+	-														
C A	2	M		1			+	+															
H R	7	M		1			+				+												
H D	11	F		3			-				+	+		-									
M C	1	F		1					-			+	+			+							
F B	24	F		1							-	-	-			+							
H J	11	M		1							-	+	+			+							
S C	2	F		1							+	+	+			+							
C W	32	M		1							+	+				+						+	
G B	1	F		1								-				+		+					
V L	7	M		1							-	-	-										+
W P	9	F		1																	-	-	+
A C	4	F	Nonparalytic	1			+		+									+					
S B	6	M		1			-												+				
B G	31	F		1					+									+					
R F	2	M		1							-	-	+										
M M	6	F		1								-	-	-	-								+

- = Stool tested negative for polio virus

+ = Polio virus recovered from stool

tinuous or intermittent affair at least insofar as demonstrable amounts of virus are concerned. As is shown in Table 128 in some patients the virus was present in every stool specimen examined whereas in other patients the virus was demonstrable in some specimens but not in others. Obviously if the virus-isolation attempts had been restricted to the examination of single specimens the results in a number of cases might well have been negative.

Data similar to those in Table 128 on the excretion pattern in poliomyelitic patients were obtained on a much larger series of patients than that presented for illustrative purposes in Table 128. Analysis of this larger body of data indicates that of the entire group of individuals shown to have been excreting virus at one time or another during the course of their disease only 75 to 80 per cent of the group excreted virus on any given day; this proportion of virus shedders appeared to hold up to approximately 30 days postonset and seemed to decrease thereafter although the rate of decline cannot be estimated with accuracy because of the small number of individuals for whom stool specimens were available after this period. These

findings like those illustrated in Table 128 point to the desirability of testing multiple stool specimens and form the basis for a collection schedule according to which a stool specimen is obtained when the patient is first seen and at 3 to 4-day intervals thereafter during the early stages of the illness. In the laboratory the specimens are tested two at a time in chronological sequence that is the first 2 stools are tested simultaneously and if the results are negative the next 2 specimens are examined; if these prove to be negative any additional specimens available are then examined when a virus is isolated the subsequent stool specimens are not examined.

Examination of multiple stool specimens favors detection of virus excretion and the value of such a procedure for this purpose is shown in Table 129. In view of the data to be presented in Table 130 it should be emphasized that Table 129 does not deal with a randomly selected cross section of the total patient population we have studied but represents a group of 231 patients on whom multiple stool specimens were available were tested and a poliomyelitic virus was recovered. It will be noted that of the

TABLE 129 RELATION OF NUMBER OF SERIAL STOOL SPECIMENS TESTED TO DETECTION OF EXCRETION OF POLIOMYELITIS VIRUS

No of Stool Specimens	No of Patients	Number of Serial Stool Specimens that were Examined before Virus Excretion was Detected						
		1	2	3	4	5	6	7
Number of Patients								
2	182	164	18					
3	24	18	3	3				
4	20	11	3	4	11			
5	4	3	0	0	0	1		
6	—	—	—	—	—	—	—	—
7	1	0	0	0	0	1	0	0
Total	231	196	24	7	2	2	0	0
Per cent	100	84.8	10.4	3.0	.9	.9		
Cumulative per cent		84.8	95.2	98.2	99.1	100.0		

This table does not include 28 virus-hedged patients from whom only 1 stool specimen was collected and 1 patient whose specimen was pooled.

Ophiopharyngitis was not included.

If 2 stools were collected on the same day after onset, they were counted as a single specimen, hence no table did the results of virus isolation tests of these pooled specimens differ.

group of 182 patients on whom 2 stool specimens were available the first specimen yielded a virus in 164 cases and examination of the second stool specimen yielded a virus in the 11 individuals in whom the first stool specimen was negative. Similarly of the 24 individuals on whom 3 stool specimens were available the virus was recovered from the first stool specimen of 18 from the second stool specimen of 3 and from the third stool specimen of the remaining

3 individuals. As is indicated at the bottom of the table the proportion of virus recoveries increased with the number of specimens examined. Thus a poliomyelitis virus was recovered from the first stool specimen of 85 per cent of this group. Examination of the second stool specimen led to the recovery of a virus from an additional 10 per cent to give a total virus isolation rate of 95 per cent. Examination of 1 additional stool specimen from those individ-

TABLE 130 VIRUS ISOLATIONS FROM PATIENTS WITH CLINICAL DIAGNOSIS OF POLIOMYELITIS

VIRUS RECOVERED	PROPORTION VIRUS RECOVERIES FROM PATIENTS DIAGNOSED AS				TOTAL PATIENTS	
	PARALYTIC POLIO		NONPARALYTIC POLIO			
	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
Polio Virus						
Type 1	173	49%	47	13%	220	31%
Type 2	4	1%	4	1%	8	1%
Type 3	29	8%	3	1%	32	4%
Types 1 and 2 and Coxsackie						
B 5 mixed	1	1%	—	—	1	1%
Other Virus						
Orphan	18	5%	102	28%	120	17%
Negative	187	37%	212	57%	339	47%
Totals	352	100%	368	100%	720	100%

This table includes patients in whom laboratory work was complete as of March 15, 1957.

All figures are from single stool specimens.

uals whose first 2 specimens were negative increased the virus-recovery rate to 98 per cent and examination of a fourth specimen further increased the rate to 99 per cent. However the number of stool specimens to be examined must be considered not only in terms of what additional information is gained in relation to the time and the effort expended but also from the practical standpoint of how many specimens one can expect to be submitted. Thus from Table 129 a virus-recovery rate of 98 per cent was associated with the examination of 3 serial stool specimens. However considerable attention occurs in the submission of multiple stool specimens and is progressively greater as the number of specimens increases.

During the interval May 1955 at which time a restricted diagnostic service was initiated through March 15 1957 we have examined stool and blood specimens from a total of 720 patients on whom both types of specimens were submitted. In most instances the stool specimens were tested in pairs as outlined above and the findings were presented in Table 130 (This table includes the results obtained with a number of stool specimens taken 30 or more days after onset some of these specimens proved to contain virus others did not.) Of the 720 individuals considered clinically to have or

possibly to have poliomyelitis the diagnosis in 352 cases was paralytic poliomyelitis and in 363 it was nonparalytic poliomyelitis either suspected or definitely stated.

Of the 352 patients with a clinical diagnosis of paralytic poliomyelitis a poliomyelitis virus was recovered from only 206 individuals or 58 per cent of the group. If a poliomyelitis virus-recovery rate of 90 per cent is accepted as an attainable goal then our virus-recovery rate is only two thirds of what it presumably could or should be. Whether or not our comparatively low isolation rate can be attributed to failure to collect stool specimens sufficiently early in the illness to improper methods of collection and handling and to shipment without refrigeration remains to be determined. However we believe that it is an accurate reflection of what can be expected in the everyday practice of diagnostic virology as applied to clinical medicine. Confronted with the realities of the situation it is obvious that adjunct diagnostic measures are required and such measures, namely serologic techniques will be considered in a moment.

With respect to the 363 individuals in whom a diagnosis of nonparalytic poliomyelitis was entertained clinically a poliomyelitis virus was recovered from the stools of only 54 individuals, or 15 per cent of the group. Proportionally this

TABLE 131 NONPOLIOVIRUSES ISOLATED FROM PATIENTS CLINICALLY DIAGNOSED OR SUSPECTED TO HAVE POLIOVIRUS

CLINICAL IMPRESSION	NUMBER OF PATIENTS	NUMBER OF PATIENTS FROM WHOM VIRUS RECOVERED WAS COXSACKIE					ECHO			NOT YET IDENTIFIED
		A 9	B 2	B-3	B-4	B-5	4	6	7&8	
Paralytic polio	19	1	3		1	2		1		11†
Nonparalytic polio	84	7	4	3	3	25‡	1		1‡	40
Suspect polio	1									1
Polio vs. Coxsackie	3					2				1
Polio vs. encephalitis	8					5				3
Encephalitis	4					3				1
Pleurodynia(?)	1					1				
Diagnosis deferred	1									1
Totals	121	8	7	3	4	38	1	1	1	58

These are the 121 patients shown in Table 130 under "Orphan" virus and mixed."

Type 1 and 2 virus also isolated from the stool of 1 of these patients.

† Type 1 virus also isolated from stool of 1 patient.

On patient had serologic evidence of Type 3 polio virus infection.

‡ In this patient, ECHO 7 virus was isolated from 1 stool. ECHO 8 from another stool.

§ Four patients had serologic evidence of polio (2 Type 1, 2 Type 2, and 1 Type 3).

nonparalytic group yielded more "orphan" viruses than did the group considered clinically to have paralysis since 107 enteric cytopathogenic viruses other than poliomyelitis were recovered from the nonparalytic group and 19 such viruses were recovered from the paralytic group; an incidence of 28 per cent and 5 per cent respectively. The 121 patients who yielded an orphan virus (Table 131) represent 17 per cent of the total group of 720 patients; this is a fairly impressive figure numerically and it becomes more impressive when one considers the time and the effort that the identification and the typing of these agents demand if they happen to be members of the Coxsackie or ECHO families. The present neutralization tests in monolayer cell cultures are cumbersome, not inexpensive and leave something to be desired. A simpler method for the identification of the Coxsackie and the ECHO groups of viruses for diagnostic purposes is highly desirable. The possibility of typing these agents by means of a reverse complement fixation test as for example can be done for identification of Western equine encephalomyelitis virus or by a colorimetric neutralization test as has been described recently for the adenoviruses merits investigation.

#### DIAGNOSIS BY MEANS OF NEUTRALIZATION OR COMPLEMENT FIXATION TESTS

For serologic purposes 2 or more serial blood specimens are requested on each patient. The specimens are always examined simultaneously in the same test. The neutralizing antibody content of the sera is determined by the method of Salk *et al.* using a monkey kidney cell system or by the method of Syverton *et al.* or of Lipton

and Steigman using HeLa or KB cell systems. Poliomyelitis complement fixation tests are performed according to the macroscopic (tube) technique described in recent publications from this laboratory. The demonstration of a fourfold or greater rise in antibody titer by either of these methods generally is accepted as diagnostically significant. As is true in general of all viral diagnostic work, the ability to detect a significant rise in titer depends on a proper temporal spacing of the serum specimens in relation to the disease process. The first serum specimen should be taken as promptly after the onset of the illness as possible (Table 132). As concerns poliomyelitis many patients already possess maximal levels of neutralizing or complement fixing antibody by the time that they are admitted to the hospital or by the time that poliomyelitis is suspected. Neutralizing antibody titers especially are apt to be at maximal levels. On the other hand the later the acute phase blood specimen is taken the greater the likelihood that antibody will be present and if there is sufficient delay maximal antibody titers will have been reached in the latter case it obviously will be impossible to detect rises in antibody levels and it will be equally difficult or impossible especially in the case of neutralizing antibody to interpret unequivocally the significance of the antibody which is present.

The value of a serologic test is not only a matter of its sensitivity in the detection of specific antibody but also a matter of the extent to which it can reveal the occurrence of diagnostically meaningful rises in antibody levels. To evaluate the practical usefulness of a test it is necessary to examine paired or multiple serum specimens from patients known to be infected and to ascertain the proportion of cases in which

TABLE 132. RELATION BETWEEN INTERVALS PROPOSED FOR COLLECTION OF BLOOD SPECIMENS AND INTERVALS ACTUALLY FOLLOWED BY PHYSICIANS SUBMITTING SPECIMENS ON 306 PATIENTS

SPECIMEN	BLOOD SPECIMENS REQUESTED	TOTAL SPECIMENS	INTERVAL BLOOD SPECIMENS COLLECTED		
			MPAN	MEDIAN	RANGE
1. Acute phase	As soon as possible after onset	306	7 days	5 days	0-30 days
2. 1st conv.	14 days postonset	25	25 days	23 days	10-60+ days
3. 2nd conv.	29-40 days postonset	103	31 days	30 days	17-60+ days†

O = specimen taken more than 60 days after onset

† F = specimen taken more than 60 days after onset

TABLE 133 DISTRIBUTION BY DAYS AFTER ONSET OF HOMOTYPIC NEUTRALIZING ANTIBODY TITERS OF 124 PATIENTS WHO EXCRETED VIRUS IN STOOLS

HOMOTYPIC NEUTRALIZING ANTIBODY TITER	DAYS BETWEEN ONSET OF ILLNESS AND COLLECTION OF SPECIMENS								TOTAL
	0-5	6-10	11-20	21-30	31-40	41-50	51-60	61-	
1 2 048			2						2
1 1 024	3	5	4	■	1	3		1	75
1 512	5	8	3	4	4	5	5	1	35
1 256	14	13	8	11	6	2	5		59
1 128	14	5	9	22	2	1	2		55
1 64	12	13	2	6		1			34
1 32	12	6	1		1				20
1 16	5	1							6
1 8	4	3							7
1 4		1							1
<1 4	2	2							4
Total	71	57	29	51	14	12	12	2	248

increases in antibody titer are detectable. Paired blood specimens from 124 patients from whom a poliomyelitis virus had been recovered were examined for their neutralizing and complement fixing antibody levels. The distribution of the neutralizing antibody titers in the acute and the convalescent phase blood specimens is shown by days after onset in Table 133.

It will be noted that the low antibody titers present from 0 to 10 days postonset were con-

verted to higher levels by 11 or more days post onset so that by the 11th day in only 2 individuals were neutralizing antibody titers of less than 1 64 encountered. It also will be noted that a rather large proportion of individuals possessed antibody titers of 1 64 or greater within the interval 0 to 5 days and 0 to 10 days postonset that is quite high and even maximal antibody levels were encountered shortly after the onset of illness.

TABLE 134 DISTRIBUTION BY DAYS AFTER ONSET OF HOMOTYPIC COMPLEMENT FIXING ANTIBODY TITERS OF 124 PATIENTS WHO EXCRETED VIRUS IN STOOLS

HOMOTYPIC C F ANTIBODY TITER	DAYS BETWEEN ONSET OF ILLNESS AND COLLECTION OF SPECIMENS								TOTAL
	0-5	6-10	11-20	21-30	31-40	41-50	51-60	61-	
1 128							1		1
1 64	2	■	11	5		2	2		27
1 32	5	5	8	15	8	5	2	1	49
1 16	5	4	4	18	4	■	5		47
1 8	5	9	3	6	1	2	1	1	28
1 4	13	7	3	7	1	1	1		33
<1 4	41	27							
Total	71	57	29	■	14	12	12	2	248

This is in contrast with the findings presented in Table 134 which shows the distribution of complement fixing antibody titers according to the interval after onset of illness at which the blood specimen was collected. It will be noted that as in the case of neutralizing antibody there was a trend toward higher antibody titers with increasing time postonset but unlike the situation with neutralizing antibody the number of individuals with high titers shortly after onset was quite small. Thus taking the interval 11 to 10 days postonset 68 serum specimens had complement fixing antibody titers of less than 1:4 as compared with only 4 sera with neutralizing antibody titers of less than 1:4.

Tables 133 and 134 illustrate in an approximate fashion because of the relatively small numbers the distribution of antibody levels that may be expected at given intervals postonset of illness. To ascertain the ability of the neutralization and the complement fixation tests to reveal increases in antibody concentration during the course of the illness it is necessary to compare the number of instances in which a significant rise in antibody titer was demonstrable. Such a comparison is made in Table 135 in which the rises in homotypic antibody (antibody

corresponding to the virus type recovered from the stool) as determined by examination of paired sera are compared. As is shown in the upper left hand portion of Table 135 no diagnostically significant change in titer occurred in 72 individuals or 18 per cent of the 124 patients in the group. On the basis that a fourfold or greater rise in antibody level constitutes a diagnostically significant increase 21 patients or 17 per cent of the group showed a rise in neutralizing antibody but not in complement fixing antibody. This is in contrast with the finding that more than twice as many individuals showed a rise in complement fixing antibody but not in neutralizing antibody; this occurred in 45 individuals or 36 per cent of the group. In 36 patients or 29 per cent of the total number of individuals a fourfold or greater rise in both neutralizing and complement fixing antibody occurred. If these figures are accumulated it will be found that 81 individuals or 65.3 per cent of the test group showed a significant rise in complement fixing antibody whereas only 57 individuals or 46 per cent of the entire group showed a diagnostically significant rise in neutralizing antibody. (These figures are of interest because of their close correspondence to those

TABLE 135 COMPARATIVE VALUE OF THE COLORIMETRIC NEUTRALIZATION TEST AND THE COMPLEMENT FIXATION TEST IN DEMONSTRATING DIAGNOSTICALLY SIGNIFICANT CHANGES IN ANTIBODY LEVEL. PAIRED SERA FROM 124 PATIENTS WHO EXCRETED VIRUS IN STOOLS

CHANGE IN HOMOTYPIC NEUTRALIZING ANTIBODY TITER	CHANGE IN HOMOTYPIC COMPLEMENT FIXATION TITER								TOTAL
	-2X	1	+2X	+4X	+8X	+16X	+32X	+64X	
4X					1				1
-2X		1	2	2	1				1
0	1	5	6	6	6	4	1		29
+2X			7	10	5	4	4	1	31
+4X	1	2	9	2	7	2	1		25
+8X	2	1	2	2	4	6	1		18
+16X			2	2	1	2	2		9
+32X									—
+64X			2			1			3
+128X					1		1		2
Total	4	9	30	24	26	19	11	1	124

4X or greater R

CF 81/124 = 65.3

N 57/124 = 46.0

CF Neut 102/124 = 82.3

obtained in another study concerned with the development and the persistence of neutralizing antibody. In that study a rise in homotypic complement fixing antibody was encountered in 66 per cent of the patients comprising the study group and a rise in homotypic neutralizing antibody was encountered in 42 per cent of the patients.) If we combine the groups in which rises in complement fixing or neutralizing antibody or both occurred we find that a rise in the titer of either antibody was demonstrable in 102 individuals or 82 per cent of the group studied.

The complement fixation test because of its simplicity and the rapidity with which results can be obtained possesses definite advantages over the neutralization test. From our experience it appears to possess the additional merit of detecting antibody rises in a greater proportion of instances than does the neutralization test, an advantage which is referable to the comparatively slow appearances and development of complement fixing antibody. By performing both complement fixation and neutralization tests on the same specimens the

proportion of patients in whom diagnostically significant antibody rises could be detected was increased from 65 per cent to 87 per cent that is by approximately one fourth. Whether or not this added advantage is worth the large amount of additional labor involved in such dual serologic examinations has yet to be determined.

Let us now consider the application of serologic tests to the diagnosis of illness in patients from whom no virus was recovered. Reference to Table 130 shows that an attempt to reach an early diagnosis through the examination of stool specimens was made in 720 patients. Of this group 352 patients were considered clinically to have the paralytic disease and 368 were considered or suspected to have the nonparalytic form. No virus was recovered from 177 patients in the former group nor from 212 patients in the latter group.

Paired or multiple blood specimens on each of the 339 patients in this virus negative group were examined to determine whether or not rises in titer of antibody had occurred to any one or more of a number of viruses. Thus the

TABLE 136 RESULTS OF SEROLOGIC TESTS ON 339 PATIENTS WITH CLINICAL DIAGNOSIS OF POLIOMYELITIS BUT FROM WHOM NO VIRUS WAS ISOLATED

SEROLOGIC DIAGNOSIS	PROPORTION OF PATIENTS WITH INDICATED LABORATORY DIAGNOSIS WHO WERE CLINICALLY REGARDED AS				TOTAL PATIENTS	
	PARALYTIC POLIO		NONPARALYTIC POLIO		NUMBER	PER CENT
	NUMBER	PER CENT	NUMBER	PER CENT		
Poliomyelitis						
Type 1	37	29%	33	16%	70	21%
Type 2	7	6%	22	10%	29	9%
Type 3	16	13%	19	9%	35	10%
Other Virus						
Mumps	12	9%	30	14%	42	12%
Herpes simplex	1	1%	1		2	
WEE*	—	—	1	1%	1	1%
SLE†	1	1%	1		2	
Dual Infection	3‡	2%	1§	+	4	1%
Diagnosis Uncertain	12	9%	14	7%	26	8%
Negative	38	30%	90	43%	128	38%
Total	127	100%	212	100%	339	100%

Table includes all patients on whom laboratory work was complete as of March 15, 1957.

\* Western equine encephalitis.

† St. Louis encephalitis.

‡ Includes 1 aseptic meningitis plus Type 1 polio, 1 case of influenza plus Type 2 polio, and 1 case of Type 1 plus Type 2 polio.

§ Type 1 polio plus mumps virus.

sera were examined for both neutralizing and complement fixing antibody levels in poliomyelitis virus and for complement fixing antibody to herpes simplex mumps Western equine and St. Louis encephalitis viruses and occasionally other viruses as indicated. The results of the serologic tests are reported in Table 136.

Of the 127 individuals with a clinical diagnosis of paralytic poliomyelitis 60 or 48 per cent showed a diagnostically significant rise in poliomyelitis antibodies. 12 individuals showed a rise in mumps antibodies, 1 a rise in herpes simplex antibodies and 1 a rise in St. Louis encephalitis antibodies. Three instances of dual infection were encountered, namely 2 cases of influenza and poliomyelitis and 1 case of dual infection with 2 poliomyelitis viruses. (The last case mentioned may represent an instance of heterotypic antibody response but is considered here to be a dual infection because of the very marked rise in antibody titer to 2 virus types.) In 12 patients the significance of the serologic findings could not be interpreted unequivocally and these cases are listed as diagnosis uncertain. In the remaining 38 individuals or 30 per cent of the group the results of all serologic tests were negative.

Of the 212 patients in whom nonparalytic poliomyelitis was suspected or on whom such a diagnosis had been made or was entertained 74 or 35 per cent gave serologic evidence of poliomyelitis. In 30 patients or 14 per cent of the group a rise in mumps virus antibody was observed and in 3 others the laboratory diagnosis was herpes simplex Western equine encephalitis or St. Louis encephalitis virus infection. 1 patient gave evidence of a dual infection with poliomyelitis and mumps viruses. In 14 patients the serologic findings were inconclusive so that a clear-cut diagnosis was not possible and in 90 others the serologic findings were negative.

### SUMMARY

In this discussion I have tried to present some of the problems encountered by a virus laboratory in connection with diagnostic work on poliomyelitis and to show how we have attempted to meet some of these problems and how certain other problems might perhaps be attacked or resolved.

Excretion of poliomyelitis virus in the stool appears to be intermittent and consequently the probability of recovering a virus is considerably enhanced if a series of stools is examined. Despite this our virus recovery rate from patients suspected to have or definitely considered to have poliomyelitis has been 58 per cent in the paralytic group and 15 per cent in the non-paralytic group. We raise the question whether or not the proportion of recoveries of the poliomyelitis, the Coxsackie and the ECHO viruses might not be increased through replacement of the widely employed monkey kidney cell cultures by conceivably a cell type with a broader spectrum of viral susceptibility or less ideally by several cell types which conjointly afford a continuum of viral susceptibility. It is possible also that the high percentage of negative results might be reduced to some extent through the use of 1-day-old mice in conjunction with tissue culture systems for virus isolation purposes.

We believe that virus isolation since this affords a means of reaching an early diagnosis should be attempted on every patient with suspected poliomyelitis on whom laboratory diagnostic assistance is desired. The significance of any agent isolated must be evaluated in terms of the patient's clinical history and course and his epidemiologic background. Failure to isolate the virus does not necessarily negate a clinical diagnosis; other laboratory approaches must be utilized. Not infrequently the diagnosis of poliomyelitis depends on serologic tests and of the 2 procedures most widely tried thus far the complement fixation test appears to be preferable to the neutralization test because of the higher proportion of cases in which diagnostically significant rises in antibody can be detected. Furthermore widespread artificial immunization against poliomyelitis threatens to impair seriously the usefulness of the neutralization test and even may affect adversely the complement fixation test to an as yet unknown extent.

Serologic tests for viral agents known to be capable of producing CNS disturbances should be included in the examination of sera from patients suspected to have poliomyelitis. Such a battery of tests is most useful when poliomyelitis virus recovery attempts as well as the poliomyelitis serology are negative and should



include in addition to tests for the mumps virus tests for those viruses known to produce CNS disease in the area or region within which the diagnostic laboratory operates

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## Discussion—Isolation of Viruses

DR ALBERT H SABIN

During the acute phase of infection isolation of virus is the most useful procedure for establishing a diagnosis of poliomyelitis. Since Dr Lennette reports failure to find virus in 25 per cent of a group of 65 paralytic patients selected as typical cases of poliomyelitis when the stools were collected early after onset and an even lower incidence of isolations in other groups one is led to wonder especially about such factors as actual time after onset that his specimens were collected, the clinical diagnostic problems and the possible inactivation of virus during shipment at specially high summer temperatures. Studies on human beings experimentally infected with attenuated strains have shown that some persons may excrete virus for only 7 to 10 days after ingestion so that with an incubation period of this duration one could expect a certain proportion of patients not to have demonstrable virus even early after onset. Such studies have also shown that the amount of virus excreted per gram of stool diminishes with time and the intermittent excretion mentioned by Dr Lennette is an indication that one is dealing with the end stage of viral multiplication. During the early stages of clinically recognized or inapparent infection 1 Gm of stool may contain more than 1 000 000 tissue-culture infective doses of virus and on several occasions we have recovered 100 000 to 800 000 infective doses from a single rectal swab.

In our own hospital and laboratory polio virus was isolated in 100 per cent of a consecutive series of 24 patients with a clinical diagnosis of paralytic poliomyelitis from whom stool or enema specimens were obtained within 2 to 8 days after onset of first symptoms—this being dated from the first relevant complaint observed by the patient or the mother. In the same group of patients we found that a single throat swab obtained 2 to 6 days after onset of first symptoms yielded polio virus in 83 per cent. A dry cotton swab was passed over the posterior pharyngeal wall several times and put into 2 ml of Hanks's solution containing 2 000 units penicillin, 2 mg streptomycin and 250 units mycostatin per ml. The swab was rubbed along the sides of the tube, the fluid was squeezed out and the material was ready for use. The results of the tests in monkey kidney cultures shown in Table 137 indicate that the incidence of positive isolations was about the same 4, 5 and 6 days after onset as at 2 and 3 days but that it was down to 50 per cent at 7 and 8 days. 5 of these 29 patients had a nonparalytic infection with polio virus in their stools. The results shown in Table 138 indicate the number of infective doses in each individual swab which ranged from 6 to 37 000. The zeros mean that virus was not found in the 0.6 ml of fluid that was tested and it is possible that if the entire

TABLE 137 EFFICACY OF THROAT SWABS FOR DIAGNOSIS OF POLIOMYELITIS  
DURING FIRST 8 DAYS AFTER ONSET OF SYMPTOMS

DAYS AFTER ONSET	NO. OF PATIENTS TESTED	NO. POSITIVE	PERCENTAGE
2-3	10	8	80
4-5-6	13	11	85
7-8	6	3	50
Totals	29	22	76

} 19/23 = 83%

21 w Type 1 1 w Type 3 only 2/29 p t t w Type 3

TABLE 138 AMOUNT OF VIRUS PER THROAT SWAB IN INDIVIDUAL PATIENTS AT DIFFERENT TIMES AFTER ONSET OF SYMPTOMS

DAYS AFTER ONSET OF SYMPTOMS	TITER OF VIRUS IN INDIVIDUAL SWABS LOG TO BASE 10					
2	4.5	3.0	3.0	1.5	1.5	0.0*
3	4.0	2.0	1.3	0.0	—	—
4	4.0	1.5	0.0	—	—	—
5	4.5	4.0	2.5	2.5	2.0	1.5
6	2.5	2.0	2.0	—	—	—
7	1.3	0.0	0.0	0.0	—	—
8	2.0	0.8	—	—	—	—

0 = None found in 0.6 ml

2 ml of swab fluid had been tested or if more than one swab had been used the incidence of positive isolations might have been still higher. The procedure of culturing polio virus directly from a throat swab or a rectal swab is so simple and the specimens are so easily obtained on admission of the patient that tests on stools could be limited only to those patients whose throat and rectal swabs fail to yield virus.

Virus isolation becomes increasingly unreliable as a diagnostic procedure as the interval after onset of infection becomes longer and in such cases the serologic tests may provide the only

means of establishing the diagnosis. While the etiologic role of certain Coxsackie and ECHO viruses in the aseptic meningitis syndrome is now well established there have been instances in which patients exhibited serologic evidence of infection with polio virus while the stools yielded only a Coxsackie or an ECHO virus. Accordingly failure to isolate polio virus from a patient exhibiting a clinical syndrome compatible with poliomyelitis does not rule out the diagnosis unless appropriate serologic tests can be interpreted as indicating that no recent infection with polio virus has occurred.

## Discussion—Neutralization Tests

DR JONAS E SALK

Dr Smith indicated that each of us is interested in another aspect of the problem of diagnosis. Our interest is in the diagnosis of immunity in poliomyelitis rather than in the diagnosis of disease. Dr Lennette has so adequately discussed the neutralization test in relation to diagnosis of disease that I will restrict my remarks

for the most part to the diagnosis of immunity. I will not go into detail in regard to the different techniques whether they be the plaque test of Dulbecco, the use of roller tubes or the color test.

First let me show you in a few slides the results of a test for immunity. In Figure 230 are shown data obtained in a study involving

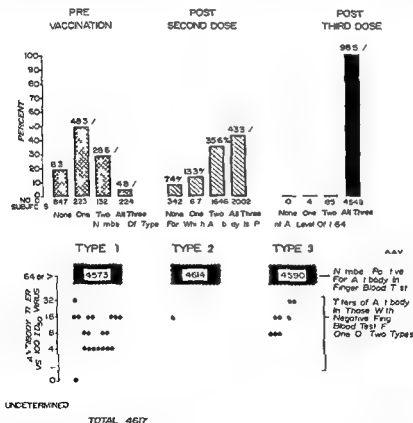


FIG. 230 (Top) Per cent distribution of individuals with antibody titers of 1:64 or greater before and after vaccination (4617 subjects given 2 doses 2 weeks apart, third dose 1 year later)

FIG. 231 (Bottom) Antibody levels determined on samples of venous blood after 3 doses of vaccine in those who gave negative finger blood test for antibody at level of 1:64

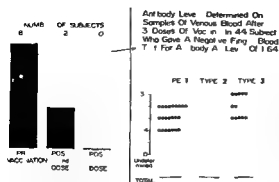


FIG 232 Number of subjects with no antibody for all 3 types at level of 1:64

4 617 individuals in whom the blood tests were done on a drop (0.7  $\mu$ l) taken from the finger whereby a test could be made of a 1:64 dilution of serum before or after vaccination. You will observe that at the 1:64 level only 4.8 per cent individuals had antibody to all 3 types and 18.3 per cent had no antibody to any type at that level. After the second dose of vaccine you will see an upward shift in the distribution of those with antibody then following the third dose given about a year later you will observe that 98.5 per cent of individuals had antibody to all 3 types at a level of 1:64 or greater.

We were then interested in the 15 per cent that did not have demonstrable antibody at the 1:64 level in the finger blood test. Venous blood was obtained and you will see (Fig 231) that in all but one instance antibody for all 3 types was present at lower levels of serum dilution. When an analysis was made merely of the 847

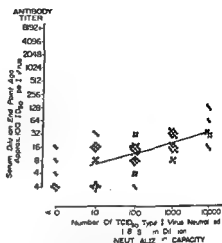


FIG 234 Correlation of two methods of serum antibody measurement: neutralizing capacity vs antibody titer (constant virus vs constant virus) serum samples from mixed group of persons after one or two doses of vaccine.

individuals who had no antibody to any 3 types those who developed antibody to all 3 types after the second or third dose. I cataloged the 44 who had no antibody to all 3 types at the 1:64 level in the finger blood test examined and you will see the results right hand portion of Figure 232.

I want to show an anomaly to indicate perhaps the desirability of having a test for neutralizing capacity. Figure 233 left depicts a child who received the inoculations at the intervals months indicated. You will see the 1 and 2 response but there seemed to be

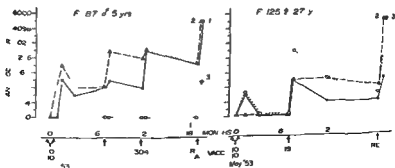


FIG 233 Response variation among individuals to the 3 antigens

# Discussion—Neutralization Tests

toriness to Type 3 until an inoculation given some 18 months later. Other individuals similarly treated responded more adequately. Figure 733 right shows another anomaly of a poor Type 1 response with a good Type 2 and 3; this too was anomalous when compared with the group as a whole.

The data in Figure 234 indicate the results of the two ways in which a test for antibody can be made and raise the question as to what is meant by the number of antibody units expressed by antibody titer as indicated on the vertical scale. Antibody measurements on the horizontal scale shown were tested using a 1:8 serum dilution and determining its neutralizing capacity against different dilutions of virus. You will observe that in some instances serum from individuals who had what appeared to be relatively low levels of antibody by the serum-dilution method shown on the vertical scale had the capacity to neutralize large amounts of virus as indicated on the horizontal scale.

The significance and the importance of this may be that perhaps we may not be measuring the significant antibody status of an individual when we measure the dilutions of serum against a constant amount of virus. What we really want to know in a diagnosis for immunity is how much virus will be neutralized by the individual's undiluted serum. Therefore a test such as the one in which perhaps a 1:8 dilution of

serum is used and is tested against different dilutions of virus may give us a better measure of immunity and of immune status; moreover technically it is far simpler to perform.

In Figure 235 is something that is purely theoretical. The word valence should have been in quotation marks. I have no way of knowing whether we are talking about valence avidity or some other factor, but when we plotted the observed relationship between neutralizing capacity of a 1:8 dilution of serum against the serum dilution end point from the data in the previous slide, a line of gradual slope was observed. If the virus increments and the antibody differences were approximately equally related, one would have expected a slope of the order of magnitude shown in the right hand insert. Therefore under the circumstances we wonder whether or not different intensities of immunization as have been observed for other immunizing agents do not alter the quality of the antibody either by way of avidity or valence so as to provide a better measure of actual immunizing effect of the serum by testing a constant dilution of serum against different dilutions of virus rather than different dilutions of serum against a constant dilution of virus.

These are random thoughts on this subject which is being studied more intensively at the present time.

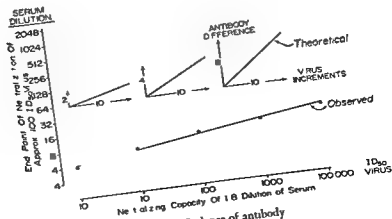


FIG. 235 Valence of antibody

## Discussion—Precipitin Test

DR G L LE BOUVIER

Precipitates can be formed by the reaction between antigens present in polio virus concentrates and antibodies present in the serum of patients convalescent from poliomyelitis or of monkeys hyperimmunized against polio virus. These precipitates are type specific. They may be observed in tests using liquid reactants alone or they may be examined by one or another of the gel-diffusion methods. I shall discuss some results obtained by the method of Ouchterlony in which the virus and serum reactants diffuse from separate cups into an agar gel contained in a Petri dish.

Monkey antiserum may produce two bands of precipitate in the agar comprising a minor as well as a major band but human sera in the tests so far performed have produced only the major band and it is this which is referred to here. The point between the virus and the serum cups at which a precipitate develops is a measure of the relative concentration of the homotypic antigen and antibody concerned. This fact makes it possible to standardize the conditions of the test and measure the polio virus precipitin content of a specimen of serum.\*

Figure 236 shows the appearance after 5 days of incubation at 37 C of a precipitin test on paired sera from 2 patients with poliomyelitis (a b and a' b'). These sera are undiluted. The center cup contains Type 3 polio virus consisting of the liquid from infected monkey kidney cultures concentrated 50 times by ultracentrifugation. On either side are cups containing different dilutions of a type specific reference serum. The precipitate formed by the higher concentration of reference serum (1/4) is further away from the serum cup and closer to the central virus cup than that formed by the lower

concentration (1/16). Both patients had a Type 1 virus infection, one of them whose sera (a' b') are seen in the upper half of the picture, possessed no detectable Type 3 precipitins; the other had about the same amount of antibody in both specimens (a' b') as is shown by the similar position and the appearance of the precipitates. The fusion of these precipitates with those produced by the reference serum argues the identity of the antigen-antibody systems involved.

Using unheated crude virus concentrates, polio virus precipitins were assayed in 60 specimens of sera. These consisted of acute and convalescent phase sera from 21 patients with either Type 1 or Type 3 virus infections and of serum collected from 9 people before and 1 month after the feeding of attenuated Type 1 polio virus.

In Figure 237 the precipitin content of each one of these sera is compared with its titer of neutralizing antibody against the same virus type, namely the one causing the infection. Each of the points represents 1 serum specimen. (The different appearances of the individual points may be disregarded in the present context.) There seems to be a general tendency for polio virus precipitating antibodies and the homotypic neutralizing antibodies to run parallel at least during the first 2 months after the onset of illness. It should be mentioned that these are total neutralizing antibodies determined by the pH test in panels and would include those described as being of low avidity in addition to the so-called "high avidity" neutralizing antibodies. A correlation has also been found during the first 2 months after infection between the precipitins detectable with crude virus concentrates and the kind of complement fixing antibodies reacting with unheated as distinct from heated polio virus.

From these findings it would appear that there is no special information to be gained from the investigation of polio virus precipitins

\* In case tests for polio virus precipitins should come to be tried out in different laboratories it may be well at this stage to enter a plea that standard reference reactants be designed and used from the start so that all such tests here or performed may be capable of being compared.

or at any rate none that would set them apart from the neutralizing and the complement fixing antibodies. Therefore even supposing that adequate supplies of virus concentrates were to be come available it seems unlikely that precipitin tests on paired sera would be adopted widely as a regular diagnostic procedure. If precipitin tests do possess any peculiar advantage it may lie in the speed with which they can provide an indication of the serum antibody content in the first days or even hours of illness. For such a purpose the sort of test described here would be less suitable than either the far swifter tube flocculation test about which Dr Churcher is going to speak or the beautifully neat and ingenious application of the agar-diffusion method about which I believe our esteemed General Chairman of the Conference will have some thing to say.



FIG 236 Appearance of a precipitin test on paired sera from 7 patients with poliomyelitis after 5 days of incubation at 37 C

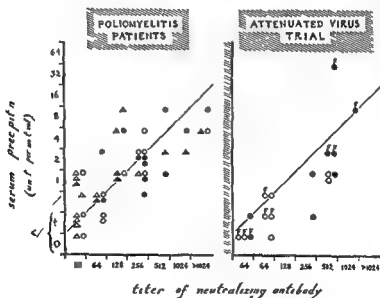


FIG 237 Precipitin content of each serum compared with its titer of neutralizing antibody (each point represents 1 serum specimen)



## Discussion—Complement-Fixation Tests

DR JOSEPH L MELNICK

Dr Lennette has adequately described the application of the microtube complement fixation (CF) test for the diagnosis of poliomyelitis. The Fulton and Dumbell plate technic also may be used and has the advantage of requiring smaller quantities of reagents. In the latter method several different concentrations of complement are used with each dilution of antigen or serum in order (1) to determine the amounts of complement fixed and (2) to give added sensitivity by a quantitative estimation of and compensation for anticomplementary factors in the reagents. However the use of the plate technic does not necessitate the use of multiple levels of complement and it may be used for 1-dimensional serum titrations similar to those described for the tube method. Both the tube and plate methods have recently been outlined in the second edition of *Diagnostic Procedures for Virus and Rickettsial Diseases* published by the American Public Health Association.

Dr Lennette has described in detail the application of the complement fixation test but a few general remarks about this test in poliomyelitis seem to be in order at a conference such as this. A strong positive complement fixation reaction may be interpreted as evidence of probable recent experience with polio virus. The complement fixation antibody response occurring in individuals who have had prior experience with polio virus of any type is usually prompt and often group reactive. Therefore it is often difficult to demonstrate an increase or to identify the type of the current infection in these individuals. However their early response to heated antigens allows one to make a tentative diagnosis in these cases on the basis of the first serum sample alone.

There appear to be 2 kinds of complement fixation antibodies formed after infection with polio virus particularly in older children and in adults who presumably have had earlier experience with one or more types of polio virus.

1. Antibodies against the group antigens are made rapidly and generally have reached their highest titer by the onset of illness, although in some patients they make their appearance soon after onset and increase in titer over the next few weeks. Exposure of polio virus to heat (or ultraviolet irradiation or formalin) uncovers a previously masked group reactive antigen common to the 3 virus types which is suitable for detecting this group antibody. By using heated antigens heterotypic fixation (against polio virus types different from that isolated from the patient) may occur in the absence of corresponding antibodies against specific antigens. These antibodies are usually absent during the acute phase of the infection and usually appear after the convalescent phase.

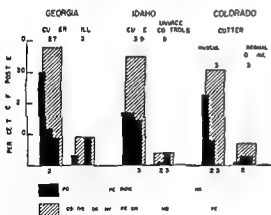


FIG. 238. Percentage of persons giving positive complement fixing response to Type 1, 2 or 3 or to any type or combination of types of polio virus after inoculation with Cutter vaccine. Controls were either unvaccinated persons in the same area or those with a vaccine other than the Cutter.

NEUTRALIZING AND COMPLEMENT FIXING ANTIBODY RESPONSE TO VACCINE P  
(1 M, 3 D y 0 7 42)

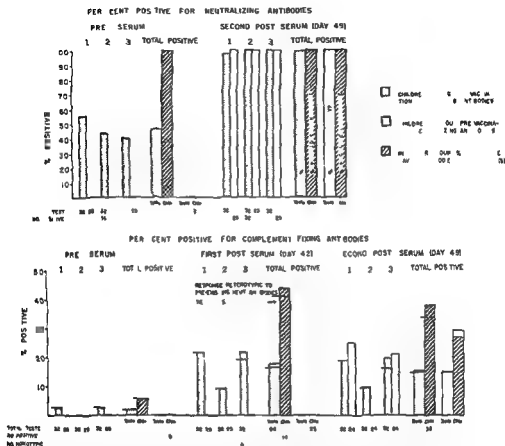


FIG. 239 Neutralizing and complement fixing antibody responses to 3 inoculations with Vaccine P by 5" children whose average age was slightly under 3 years. Responses to each type of polio virus are shown by dotted columns for the children who had neutralizing antibodies to some type before vaccination and by white columns for the children who had no neutralizing antibodies to any type before vaccination. The cross-hatched columns show the per cent of children having antibodies to any type in the serum taken on the day indicated.

Our recent experiments have shown that the complement fixation antigen unit is only 8 m $\mu$  in diameter in contrast with the diameter of the infective particle which is 27 m $\mu$  in diameter. These measurements were obtained as mentioned earlier at this conference by subjecting polio virus to ionizing radiation and determining the relative rates of inactivation of infective virus and the complement fixation antigen. The data suggest that within the virus

particle there exist several complement fixation antigenic units.

To date the response with the complement fixation antibody response in vaccinated children suggest that both dead and live viruses can elicit complement fixation responses but the level of response after infection appears to be much greater than after vaccination with killed virus. First I would like to show the data obtained in the spring of 1953 when live virus



quency of response as those inoculated with Cutter vaccine in other parts of the country. However, children receiving one tenth of this dose (intradermally) had a small percentage of positive reactions. The explanation may lie in the fact that this is an example of titrating live virus in children close to the end point of infectivity. Thus with 1 ml the frequency of response was 33 per cent and with 0.1 ml 8 per cent. On the other hand the route of inoculation may play a role for small amounts of virus introduced intradermally may produce less trauma and the virus may not multiply as readily as if the inoculation were intramuscular.

The results in 1955 shown in Figure 238 were obtained with one dose of vaccine and from our observation at least the vaccines other than Cutter were not of unusually high potency. Other observations that we have made with vaccine of unusually high potency are illustrative of the kind of response that one may get with a killed vaccine. Figure 239 shows that children receiving vaccine P (3 doses on days 0, 7 and 47) had responded by the 47th day with respectable levels of neutralizing antibodies in virtually all the children. The group studied consisted of 51 young children whose average age was slightly under 3 years so that the neutralizing antibody responses, particularly those in the 25 triply negative children, are impressive.

The complement fixation responses in this group are also shown in Figure 239 where the shaded columns pertain to the 37 children who had some neutralizing antibodies in their pre-

vaccination specimens. Only 1 child among the 32 had complement fixation antibodies before vaccination. Following 2 doses of vaccine antibodies appeared in 10 to 22 per cent of the children who had prior neutralizing antibodies but not one of the triply negative children responded at this time. Following the booster dose on the 42nd day complement fixation antibodies appeared in as many as 25 per cent of these triply negative children suggesting that the previous inoculations had sensitized them to the antigen.

Similar data for 78 children inoculated with another potent vaccine (Q) are shown in Figure 240. Again the first children to respond with complement fixation antibodies come from those possessing neutralizing antibodies prior to vaccination.

These data are similar to those which we had observed 3 years ago when we studied the response of monkeys to formalinized vaccine. Even though the complement fixation response after vaccination seems to be transitory its presence must make one extremely cautious in interpreting the results of a single serum specimen. If only single specimens are available it is obvious that large numbers of controls collected at the same time must be studied before one can place an interpretation on the laboratory data.

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# Discussion—Precipitin Test

DR. GILLIAN CHURCHER

The serologic tests available at present for the diagnosis of poliomyelitis have their limitations as Dr. Lennette has emphasized. The ideal test would be simple, rapid, positive at a very early stage of infection and strictly type specific. There is some prospect that this ideal may be realized largely by a test based on the direct virus flocculation reaction which we described recently.

The reaction was first demonstrated with virus concentrates and the sera of artificially immunized rabbits by the standard Dreyer tube method used for bacterial agglutination. This is certainly the method of choice for diagnostic application, but the need for economy of reagents has compelled us to evolve a micro method using dark ground illumination for observation of the reaction. This in spite of several disadvantages has had to be used for all our subsequent investigations.

In extending our experiments to the investigation of human sera we have continued to use rabbit antisera for the testing and the standardization of antigen batches. The reaction with rabbit antisera has always been strictly specific.

To gain some understanding of the flocculation pattern given by human sera we have titrated them by the chessboard technic. The results of titrating a pair of sera taken from a Type 1 case from whom a virus was isolated

is shown in Table 139. The sera were taken on the 4th and 26th days after the onset of symptoms. In this case heterologous reactions occurred to low titer. Some of the human sera have shown no heterologous reaction. Because of the early appearance of flocculating antibodies found in many of these sera it seemed important to obtain some idea of the flocculation titers of normal sera. A number of sera from healthy adults were tested and in only one instance was flocculating antibody present. In fact several of these sera did have neutralizing antibodies to one or more types of polio virus. Therefore we provisionally accepted the presence of even small amounts of flocculating antibodies as being indicative of current or recent infection. A small survey of sera from cases in which virus isolations have been made has shown that flocculating antibodies appear in a high proportion. This is demonstrated in Table 140 which shows the presence of flocculating antibodies in sera from Type 1 and Type 3 infections. The right hand column shows those instances where the homologous flocculation titer exceeded any heterologous reaction.

Table 141 shows the correlation between flocculation and complement fixation results. With the proviso that the figures are far too small to permit significant statistical analysis it appears that the flocculation test gives a much higher percentage of correct diagnosis than does the

TABLE 139. POLIOMYELITIS FLOCCULATION REACTION. CHESSBOARD TITRATIONS OF SERA FROM TYPE 1 PARALYTIC CASE

BRUN ANTIGEN	4TH DAY SERUM								26TH DAY SERUM						ANTIGEN SALINE CONTROLS
	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/4	1/8	1/16	1/32	1/64	1/128		
1/2	+++	+++	++	++	—	—	—	+++	+++	++	+	—	—	—	—
1/4	+++	+++	++	++	+	—	—	+++	+++	++	+	—	—	—	—
1/8	++	++	+	+	±	—	—	+	++	+	+	—	—	—	—
1/16	±	+	+	+	+	—	—	—	±	+	+	±	—	—	—
1/32	—	±	±	±	±	±	±	—	—	±	±	±	±	—	—
1/64	—	—	—	±	±	±	±	—	—	—	±	—	—	—	—

TABLE 140 POLIOMYELITIS DIAGNOSIS FLOCCULATION TESTS OF HUMAN SERA

VIRUS ISOLATED	FLOCC VS ANTIGENS			HOMOLOGOUS TITER HIGHEST
	1	2	3	
Type 1	8/10	3/8	4/10	5/8
Type 3	2/6	2/5	5/6	5/5

N me at = Numb po t e

D m nat = Numb r t sed

TABLE 141 POLIOMYELITIS DIAGNOSIS CORRELATION OF FLOCCULATION AND COMPLEMENT FIXATION RESULTS

TYPE OF CASE	VIRUS TYPE ISOLATED	NUMBER OF SERA SHOWING				TOTALS
		F+ C+	F+ C-	F- C+	F- C-	
Paralytic	Type 1	4	3	0	0	7
Nonparalytic	Type 1	4	1	0	1	6
Paralytic	Type 3	2	3	0	3	8
Paralytic	Nil	0	2	0	1	2
Totals		10	9	0	4	23

complement fixation test. In no case was complement fixation positive when flocculation was negative though the reverse was frequently the case.

There are innumerable problems still to be solved before adequate correlative serologic surveys can be undertaken. Among others these involve preparation, standardization, stability, storage, and inactivation of the virus antigens. It appears though that the early appearance of flocculating antibodies before the appearance of complement fixing—or a significant rise in neutralizing antibodies—may be one of the most valuable features of this test, but it should be stated quite clearly that we make no claim

that flocculation provides as yet a reliable diagnostic test—merely that it may do so in the near future.

Acknowledgments are due to Dr Alastair Dudgeon and Dr Ann Peach of St George's Hospital Medical School London SW 1 who supplied the sera of poliomyelitis cases with the results of complement fixation and neutralization tests.

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## DISCUSSION

PROF DR BERNKOPF I shall confine myself to a short report of our experience in the isolation of polio and other enteric viruses for diagnostic purposes. As Dr Lennette has stressed the choice of a suitable cell system is of primary importance. We have used human amnion cells extensively and have found them to be highly satisfactory. The ready availability of placentas everywhere and the simple methods needed for the preparation of the cultures make the human amnion useful for many purposes. There should be no difficulty in having such cultures prepared in any normal hospital laboratory as no special equipment or facilities are required. The number of placentas which do not furnish suitable cells is extremely small if a few simple rules as to pH and temperature are adhered to.

The susceptibility of amnion cultures for polio and ECHO viruses is high and compares favorably with monkey kidney and HeLa cell cultures. A number of Coxsackie B and A9 strains also do well in them. The high susceptibility of these cultures for polio viruses may be seen from the fact that in a series of 102 cases of paralytic poliomyelitis from which only single stool specimens were obtained 88 cases yielded a cytopathogenic agent 75 of them polio viruses in which from 13 cases agents were isolated in amnion cultures which were not neutralized by polio antisera some of them apparently mixtures of polio virus with other cytopathogenic agents. These mixtures of several viruses represent a special diagnostic problem.

The high susceptibility of amnion cultures for other enteric viruses may be seen from the fact that among 440 stools collected from a normal child population at different seasons of the year no less than 45 per cent of the specimens contained cytopathogenic agents. Polio viruses accounted for only 35 per cent of them the rest were mostly Coxsackie and ECHO viruses or mixtures of them. It is obvious that under these conditions it is extremely difficult to evaluate the diagnostic significance of cytopathogenic nonpolio agents isolated from the stools.

We have paid special attention to the possibility of using differences in the cytopathologic

effects caused by the various virus types in amnion cultures for a preliminary differential diagnosis. If infected cultures are examined with low power stained or unstained in the culture tubes before the later stages of degeneration set in it is frequently possible to arrive at a tentative identification of polio Coxsackie or ECHO virus types by using the morphologic changes only. Of course it is understood that final identification always must be based on the neutralization of the cytopathogenic effect by specific antisera. With this reservation it may be said that a number of characteristic differential features differentiate the changes caused by the various virus types.

Time does not permit giving a detailed description of these changes. I shall mention only the fine ramifications of cell processes which appear with great frequency in polio-infected cells while they are absent or rare following Coxsackie or ECHO virus infection also a sharp separation of ecto and endoplasm which is characteristic for certain Coxsackie strains and the delayed or unequal changes following ECHO virus infections in which completely normal looking cells persist for extensive periods next to severely damaged elements. Completely different characteristic pictures are produced by adenoviruses herpes virus and others. It seems to us that these differences in cytopathology produced by various virus types in tissue culture have a potential value in diagnostic work which has not yet been fully exploited.

PROF BLASKOVIC "Dr Lennette's paper described the situation prevalent in practice emphasizing all the difficulties in determining the proper diagnosis of patients suffering from a virus infection of the central nervous system. This holds true mainly in the group of nonparalytic infections simulating poliomyelitis. Analyses made by Dr Lennette have shown the necessity of extending the examination on the group of neurotropic viruses which are common in a particular region or country and can simulate poliomyelitis.

In Czechoslovakia differential diagnosis of

poliomyelitis is made in relation to tick borne encephalitis mumps lymphocytic choriomeningitis and the Coxsackie group. The diagnosis of ECHO infections has not been carried out in central laboratories until now nor has sufficient attention been paid to the primary infection of the central nervous system with herpes simplex virus.

As I mentioned attention is paid in the diagnosis of tick borne encephalitis particularly since the transmission of the infection was confirmed epidemiologically by the tick bite and by milk of infected goats and cows as well. It was shown experimentally that the virus is excreted by milk when some days previously a virus suspension had been administered subcutaneously or intradermally as is shown in the following tables.

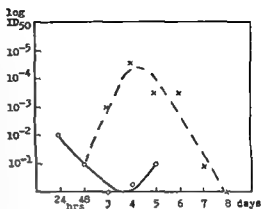


FIG 741 Virus concentration in blood (—) and in milk (---) of experimentally infected goat

TABLE 142 COMPLEMENT FIXATION TESTS OF PATIENTS SUSPECTED OF INFECTION WITH TICK BORNE ENCEPHALITIS (CENTRAL MORAVIA)\*

YEAR	SAMPLES	NO OF TESTED PERSONS	NO OF SAMPLES	
			POSITIVE	NOT SUITABLE
1953	569	445	85	9
1954	756	393	138	14
1955	733	505	76	17
Total	2 058	1 343	299	40 (1.44%)

\* Tick L. aff. tick L. 7th Imm. nat. f. sch. 113 761 1956

TABLE 143 ISOLATION OF TICK BORNE ENCEPHALITIS VIRUS (CZECHOSLOVAK STRAIN) FROM BLOOD MILK URINE AND STOOL OF EXPERIMENTALLY INFECTED GOATS

No OF ANIMAL	MATERIAL	INTERVAL AFTER INFECTION								
		6 Hrs	24 Hrs	48 Hrs	3 D	4 D	5 D	6 D	7 D	8 D
180	Blood	●	●	●	●	●	●	—	—	—
	Milk	—	—	●	●	●	●	●	—	—
	Urine	—	—	—	—	—	—	—	—	—
	Stool	—	—	—	—	—	—	—	—	—
876	Blood	●	●	●	●	—	—	—	—	—
	Milk	—	—	—	●	●	●	●	—	—
875	Blood	—	●	●	●	●	—	—	—	●
	Milk	—	—	●	●	●	●	●	●	—
184	Blood	—	●	●	●	●	—	—	—	—
	Milk	—	—	—	—	—	●	●	●	—

● = Isolated  
— = Not isolated



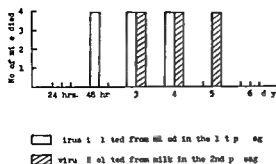


FIG. 242 Isolation of tick borne encephalitis (Czechoslovak strain) virus from blood and milk of experimentally infected cow

Table 142 shows surveys of samples collected in a laboratory of central Czechoslovakia in 1953 1954 and 1955. You see the positive results of samples in each year. Now let us consider virus distribution of tick borne encephalitis in the blood and the milk of experimentally infected animals.

Table 143 shows the viremia after the infection of goats. Virus was found in the blood until the fifth day and was excreted in the milk from 48 hours to 8 days. Figure 241 shows the virus present in milk and blood of the goat when estimated in titer. As is seen it reaches titers which are quite high.

The same experiments were run in sheep and you can see (Table 144) a similar picture.

If the cow is infected (Figure 242 shows the first experiment) the virus could be detected

not only from blood but also in the milk for a couple of days.

We are paying attention to this infection because it is sometimes rather difficult to differentiate clinically the polio and the tick borne encephalitis infection in sporadic cases. The seasonal occurrence of tick borne encephalitis lasts from April to October and reaches its peak in July and August when polio cases occur.

I shall not speak of the diagnosis of polio by virus recovery and complement fixation tests. It is done by methods which are used elsewhere. I shall mention only that in the Prague laboratory more than 100 polio viruses were recovered from stool specimens and the same holds true for the differential diagnosis of lymphocytic choriomeningitis which has been established in our country since 1949. Of Coxsackie viruses 13 strains of different types were recovered in our laboratory last year.

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TABLE 144 ISOLATION OF TICK BORNE ENCEPHALITIS VIRUS (CZECHOSLOVAK STRAIN) FROM BLOOD AND MILK OF EXPERIMENTALLY INFECTED SHEEP

SHEEP No	MATERIAL	INTERVAL AFTER INFECTION								
		24 Hrs	48 Hrs	3 D	4 D	5 D	5 D	7 D	8 D	9 D
867	Blood	—	●	●	—	—	—	—	—	—
	Milk	—	●	●	⊕	⊕	—	—	—	—
868	Blood	●	●	●	●	—	—	—	—	—
	Milk	—	—	—	—	—	—	—	—	—
869	Blood	●	●	●	●	●	—	—	—	—
	Milk	—	●	⊕	●	●	●	⊕	—	—

● = Virus isolated in the 1st passage

⊕ = Virus isolated in the 2nd passage

— = No virus isolated

DR GARD Like Dr Salk I would like to comment on the diagnosis of immunity rather than the diagnosis of infection

Inactivation of virus by antibodies is a much slower process than usually is realized. The reaction rate is highly temperature-dependent and is not the same in all virus antibody systems.

In a mixture of polio virus and human or guinea pig immune serum the full effect is not attained until after about 6 hours at 37 C at refrigerator temperature after about a week. In the conventional neutralization test where the virus serum mixture is kept at room temperature for 30 minutes or an hour the reaction has hardly even begun. What is measured by this test is mainly the effect of antibody carried over into the test medium—experimental animals or tissue cultures—and at the level of the final dilution in the respective medium. Therefore the conventional test is not suited for a demonstration of low levels of serologic immunity.

An average thirtyfold increase in sensitivity can be achieved if the reaction between virus and antibody is allowed to proceed to completion before the reaction mixture is seeded into the test medium. This technique (immuno-inactivation) as distinct from the conventional test will be described in detail in a forthcoming paper.

Immuno inactivation may not be of particular importance in routine diagnostic work. However it fills a purpose in the search for traces of antibody as an indicator of previous exposure to the virus. Thus in the evaluation of effectiveness of vaccines it is of great importance that the existence of preinoculation immunity can be excluded which according to our experience is by no means possible with the aid of the conventional test. So far we have been unable to find a case in which so-called hyperreactivity was not associated with the presence of antibodies demonstrable by a sufficiently sensitive technique. In particular appearance of heterologous neutralizing antibodies in the course of natural infection or during monotypic artificial immunization was observed only when a pre-existing low level heterologous immunity was demonstrable. I would like to add also that the concept of valency of antibodies or avidity of antibodies ought to be considered after studies in

which proper attention was paid to the time relationships of the neutralization action.

DR C FAR One of the objects of the Polio Research Foundation of Southern Africa was to provide a service for the diagnosis of poliomyelitis. This service now provides for the Union of South Africa the territories of southern Africa on occasion for countries farther afield and also the South Sea Islands. From 1945 to 1957 monkeys were used for these tests. They are freely available in South Africa but even so the number of tests which could be undertaken was limited. Since 1952 the tissue-culture technique has been applied to this purpose and with its introduction a considerable expansion in the scope of study was possible. It has been our aim to establish the diagnosis of every notified case of poliomyelitis. In particular we have studied the cases admitted to the Johannesburg Fever Hospital with a diagnosis of poliomyelitis and have worked in close collaboration with Dr Jackson the senior physician. Our laboratory staff concerned know personally every case which they study. Such collaboration between the physician and the laboratory worker as Dr Lennette has emphasized is essential for best results.

As our knowledge has increased expansion of the facilities has progressively increased too and the following teams have now been established to meet the needs the polio virus team the Coxsackie virus team and the ECHO virus team. These teams of course are necessary because the diseases caused by these groups of viruses the Coxsackie virus and the ECHO virus may simulate paralytic poliomyelitis on occasion and more often nonparalytic poliomyelitis. Then there is an arthropod borne virus team because many of the arthropod borne viruses may simultaneously simulate poliomyelitis. So may animal diseases transmissible to man because on occasion rabies may simulate bulbar poliomyelitis. Then there is the serology department which is responsible for the neutralization tests the complement fixation test and perhaps in the future the precipitin test.

In addition to these virus teams the facilities of bacteriologic hematologic pathologic and histology departments have also proved to be necessary. Such an approach demands a large organization and only a large institution with

TABLE 145 POLIO VIRUS ISOLATIONS AND TYPING OF STRAINS  
FROM THE UNION OF SOUTH AFRICA 1955-57

YEAR	POLIO VIRUS TYPES			Total
	1	2	3	
1955	77	5	48	130
1956	655	25	50	730
1957 (Jan-Apr)	376	5	16	397
Totals	1108	35	114	1257

all these facilities can undertake such a comprehensive service

The results obtained have been of some interest. Apparently the service is not yet comprehensive enough because in the investigation of an outbreak of fever which occurred at Durban in spite of a detailed study of many cases the etiology was not found. I wonder whether or not it would be necessary to set up a department of psychological medicine.

In the recent epidemics of poliomyelitis over 1 000 virus strains were isolated. I will not give the results in detail (see Table 145). However it is of some interest to note that our isolation rate appears to be much higher than that reported by Dr Lennette. In particular mention might be made of the studies carried out in cases occurring in the city of Victoria investigated in collaboration with Dr Nelson. Of 83 cases studied there virus was isolated 100 per

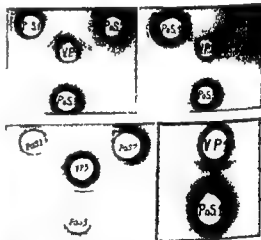


FIG 243 (Left) Microscopic slide showing how holes of 2 mm in diameter are punched out in a solidified layer of 1 per cent agar solution (Top center) Two precipitation lines obtained with polio virus Type 1 (Strain 6186) when reference monotypic sera are used (Top right) Two precipitation lines obtained with polio virus Type 2 (Strain MEF<sub>1</sub>) when reference monotypic sera are used (Bottom center) Single precipitation line obtained with polio virus Type 3 (Saukett strain) when reference monotypic sera are

used (Bottom right) Difference in intensity when 2 precipitation lines are observed (as in the case of polio virus Types 1 and 2)

cent. Perhaps the higher isolation rate can be related to the more virulent more cytopathogenic strain of virus responsible for the recent epidemic.

Also it is of interest to note that in an outbreak in which one case occurred in a nursery school 70 per cent of the other children were found to be infected. It may be of some interest to report that in 1954 Coxsackie Group B Type 4 virus was isolated from the cerebrospinal fluid of a number of cases diagnosed as nonparalytic poliomyelitis.

The following summer (in the Northern Hemisphere) the same virus was isolated in Holland. In 1955 we isolated from the cerebrospinal fluid of cases a virus which we called a new type Coxsackie A virus. Subsequently this virus was shown to be identical with the ECHO Type 9 virus responsible for the extensive epidemic in Western Europe and North America. It seems that it would be worthwhile to trace the spread of infection from one area to another from one country to another and possibly from one hemisphere to another. It seems worthwhile that international arrangements should be made in the same way as they have been made in the case of influenza and poliomyelitis to enable this to be done. Perhaps the International Poliomyelitis Congress may undertake that responsibility.

**PROF. GRASSET.** For the study of the antigenic constitution of the different types of polio viruses and the research of precipitating antibodies in sera the author used the Ouchterlony method of immunoprecipitation. Because the usual technique with Petri dishes requires too large quantities of viral antigen for routine use we have elaborated a microtechnique of double diffusion in agar gel which necessitates only very small amounts of material. Holes of 2 mm in diameter are punched out 3 to 4 mm apart in a thin layer of a 1 per cent agar solution (solidified on the surface of a routine microscopic slide) (Fig. 243 *left*). Into separate holes are introduced respectively 0.005 ml. of serum under test and 0.005 ml. of a high titer polio virus suspension (obtained from culture of infected B cells) concentrated 100 times by ultracentrifugation. The double diffusion results in the formation of precipitation lines in the agar when the serum contains antibodies specific to

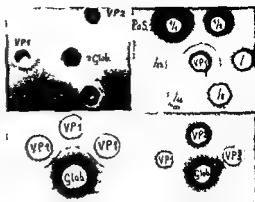


FIG. 244 (*Top left*) Precipitation of the 3 types of polio virus by gamma globulins (*Top right*) Slide illustrating how it is possible to evaluate the precipitation rate of the serum by introducing serum in decreasing concentrations into holes surrounding the central hole containing the viral antigen (*Bottom left*) Continuous precipitation line when the same antigen is disposed in the 3 adjacent holes (*Bottom right*) Precipitation lines crossing each other when 3 different types of antigen are examined.

the polio virus type introduced into the reaction. The lines appear after 18 to 24 hours at 20°C. The preparations can be dried and stained and thus can be kept as documents.

When reference monotypic sera are used in this system 1 or 2 precipitation lines are observed with polio virus Type 1 (strain 6186) (Fig. 243 *top center*), 2 precipitation lines with polio virus Type 2 (strain MEF<sub>1</sub>) (Fig. 243 *top right*), and a single line with polio virus Type 3 (Salkett strain) (Fig. 243 *bottom center*) when these are confronted with their respective monotypic serum.

When 2 precipitation lines are observed (as in the case of polio virus Types 1 and 2) they differ in intensity (Fig. 243 *bottom right*).

From these experiments it appears that the precipitation reactions are type specific and thus can be used either for the typing of poliomyelitis viruses or for the detection of specific precipitating antibodies.

Gamma globulins of human origin as well as sera from rabbits, guinea pigs and human beings vaccinated with the Salk vaccine precipi-

tate in most cases the 3 types of poliomyelitis (Fig 244 top left)

Sera from nonimmunized rabbits and guinea pigs give no reaction with polio virus antigens

When serum in decreasing concentrations is introduced into holes surrounding the central hole containing the viral antigen it is possible to evaluate the precipitin rate of the serum (Fig 244 top right)

Furthermore the technic permits comparison of 1 or more viral antigens on the same slide

Figure 244 bottom left shows a continuous precipitation line when the same antigen is disposed in the 3 adjacent holes. On the other hand Figure 244 bottom right shows precipitation lines crossing each other obtained when 3 different types of antigens are examined

The assays made to separate the antigenic constituents of diverse types of polio virus by electrophoresis in agar did not demonstrate the presence of constituents other than those already observed

The precipitin microreaction in agar has the following characteristics and advantages

1 The reactions obtained are specific. Precipitating antibodies of a serum are brought into evidence; the typing and the titer of these antibodies are determined in less than 24 hours

2 The method is simple and can be performed rapidly. The live antigen by its preparation can be conserved for many months in refrigeration without any adjuvant and thus is ready for immediate use

3 Minute amounts of material are used (0.005 ml of antigen and of serum). Up to 6 different sera can be tested on 1 slide (6 holes

containing the sera are disposed around a seventh containing the antigen)

4 The preparations dried and stained on the slides can be kept indefinitely for documentary evidence

Studies are being carried out to determine the relationship existing between the precipitating antibodies thus brought into evidence and the antibodies demonstrated by the seroneutralization tests

Dr JOHANSSON: As we heard from Dr Sved myr the other day there is a heterologous antibody response in patients' sera as regards polio virus and ECHO virus Type 6. This seems to have a general validity for enteric viruses.

Table 146 shows how complement fixation antibodies against ECHO 6 virus using a concentrated living antigen from monkey kidney tissue fluid were found in 92 per cent of patients if homologous virus was isolated from feces. If no virus or heterologous virus was recovered antibodies were found also in 92 per cent and in 2 out of 3 patients from whom Coxsackie B4 was isolated there was a significant rise in complement fixation antibodies against ECHO 6 antigen. These observations were an indication for further study of this phenomenon. Table 147 shows how in the sera of B3 excretors there was a fourfold or greater rise in complement fixation antibody titer against ECHO 6 in 73 per cent.

The same kind of heterotypic antibody response was also found between different ECHO types. Table 148 lists a series of complement fixation tests on paired sera from virus positive

TABLE 146 OCCURRENCE OF NEUTRALIZING AND COMPLEMENT FIXING ANTIBODY RESPONSE TO ECHO VIRUS TYPE 6

DIAGNOSIS	TYPE OF AGENT ISOLATED	NUMBER OF PATIENTS WITH ANTIBODIES OF TOTAL NUMBER EXAMINED		NUMBER OF PATIENTS WITH SIGNIFICANT RISE IN TITER	
		NEUTRALIZATION	COMPLEMENT FIXATION	NEUTRALIZATION	COMPLEMENT FIXATION
Aseptic meningitis	ECHO 6	36/36	30/38	14/18	7/13
	Coxsackie A 7	0/2	1/2	0/0	0/0
	Coxsackie B-4	3/1	6/6	0/4	2/3
	No virus isolated	6/14	11/12	4/6	1/4
Others	ECHO 6	2/2	3/4	1/1	0/1
	No virus isolated	5/18	16/17	2/8	0/5

TABLE 147 OCCURRENCE OF COMPLEMENT FIXING ANTIBODY RESPONSE TO ECHO 6  
IN PATIENTS FROM WHOM COXSACKIE B 3 WAS ISOLATED

CASE NO	ONSET	DATE OF SERUM	CF TITER AGAINST ECHO 6	NEUTRALI- ZATION TITER AGAINST COXSACKIE B-3	DATE OF SERUM	CF TITER AGAINST ECHO 6	NEUTRALIZATION TITER AGAINST COXSACKIE B 3
CV 1073	11/3/1950	9/15					
CVI 933	8/20/1952	8/19	4	18	11/29	64	24
CVII 40	8/25/1952	8/27	4	<10	9/1	2	25
CVII 41	8/25/1952	8/27	2	<10	9/13	16	25
CVII 42	8/25/1952	8/27	16	<10	9/13	16	15
XII 68*	8/25/1952	8/31	16	10	9/16	16	25
XII 87	8/30/1952	9/1	16	17	9/13	64	21
I 246	9/2/1952	9/5	2	<10	9/13	16	20
J 13	9/15/1952	9/17	32	10	10/1	8	23
II 189	9/24/1952	9/24	16	27	10/15	32	19
II 247	9/28/1952	10/4	8	15	10/16	32	23

N g a t i n h m n m b y n c l g u l t u r e  
 V e g t l g e l u l d d g t o R d d M n h

TABLE 148 THE OCCURRENCE OF FOLDFOLD RISES OR GREATER IN COMPLEMENT FIXING  
ANTIBODY RESPONSE IN VIRUS POSITIVE PATIENTS AGAINST 6 DIFFERENT VIRUSES

VIRUS ISOLATED	ECHO 4	ANTIGENS USED IN ECHO 6	COMPLEMENT FIXATION TESTS ECHO 9	COXSACKIE B 3	COXSACKIE B-
ECHO 4	8/11	5/11			
ECHO 6		10/20			
ECHO 9		6/15	1/17	4/14	7/11
Coxsackie B 3		9/13	8/14	6/14	3/14
Coxsackie B 4	5/10	6/11	0/12	3/12	3/14
Coxsackie A 7	1/1	2/8	6/11	4/11	1/12
			0/8	0/1	6/11
					1/8

TABLE 149 THE OCCURRENCE OF FOLDFOLD RISES OR GREATER IN COMPLEMENT FIXING  
ANTIBODY RESPONSE AGAINST 1 UP TO 5 DIFFERENT ANTIGENS

VIRUS ISOLATED	1 ANTIGEN					2 ANTIGENS	3 ANTIGENS	4 ANTIGENS	5 ANTIGENS
	HOMOLOGOUS	HETEROLOGOUS							
ECHO 4	2	3				2	3		
ECHO 6	3	2				3	0		
ECHO 9	4	3				1	0	1	
Coxsackie B 3	0	6				4	3	2	
Coxsackie B 4	0	2				4	0	0	
Coxsackie A 7		1				0	1	2	2
						0	1		

patients and Table 149 indicates the occurrence of fourfold or greater rises in complement fixing antibody response against from 1 to 5 different antigens. Notice that in sera from 113 excretors where only 1 antigen gave a significant antibody response this in all cases was heterotypic. In 2 B3 excretors there was a significant rise against 5 antigens. These 2 cases also have a significant rise against all 3 polio virus types.

I leave the question open as to whether this phenomenon depends on the occurrence of a common antigen for enteric viruses or on an amnestic reaction or on some other factors. In any case simultaneous infections can be excluded.

Obviously complement fixation tests with antigens of this kind are useless for diagnostic purposes as regards enteric viruses and we must rely on isolation of virus.

DR LIKAR: I would like to give a short account of results obtained, difficulties encountered and mistakes made in a routine virus laboratory covering an area of 1.5 million inhabitants in the northern part of Yugoslavia called Slovenia. The results refer to 157 patients in whom the final clinical diagnosis was paralytic poliomyelitis and to 700 cases of aseptic meningitis. All the cases were reported between January 1, 1955 and January 1, 1957. Emphasis will be laid on a comparison of the etiologic agents in the various neurotropic virus infections in 1955 and 1956. The first of these was a nonepidemic year while the second was that during which the greatest poliomyelitis epidemic yet recorded occurred.

Initially serologic methods and isolation techniques for the detection of infections with LCM, herpes simplex, mumps and Russian spring summer meningo-encephalitis viruses were used. At the beginning of this survey isolation procedures for poliomyelitis viruses and some other cytopathic agents were added and performed routinely.

Fibroblasts grown from minced human embryonic skin and muscles and in a few experiments trypsinized human amniotic membranes were used for the isolation of cytopathic agents. In many instances material was inoculated both in suckling mice and tissue culture but as the source of suckling mice was limited we attempted the virus isolation on suckling mice only on request of clinicians.

Some experiments were made to test our technique of virus isolations with the optimal material for the primary isolations obtained in 1956 from brain of fatal cases. The influence of repeated attempts of virus isolations and subpassages, the size of inoculum and that of cortisone were investigated.

From 31 specimens 6 were found to be positive for polio virus at the first attempt for virus isolation. Repeated subpassages of negative specimens did not prove to be very helpful but repeated first inoculations of material were found valuable for 5 strains were isolated on the second attempt for isolation from the specimens already tested and found negative for virus. In 2 cases larger inocula—1.0 ml instead of 0.1 ml—gave evident cytopathic changes 2 to 3 days earlier but no new strains were isolated using larger inocula. Addition of cortisone did not show any visible influence on the virus or tissue cultures in doses up to 0.25 mg per ml of the growth medium.

In Table 150 the final results of polio virus isolations from brain of fatal cases are given. In a great majority the strains were isolated when the patient died with the diagnosis paralytic poliomyelitis. A small number of cases of sudden death were investigated for the presence of polio viruses as they occurred at the time of the outbreak of poliomyelitis and were suspected by clinicians to be poliomyelitis. In one case when the patient died with the diagnosis poliomyelitis it was subsequently established that the death was accidental and caused by an electric shock.

TABLE 150. POLIO VIRUS ISOLATED FROM BRAIN OF FATAL CASES IN 1956

PARALYTIC POLIOMYELITIS		ENCEPHALITIS		SUDDEN DEATH	
No Pos	No Neg	No Pos	No Neg	No Pos	No Neg
10	4	1	5	0	8

TABLE 151 VIRUSES ISOLATED IN 1955

CLINICAL DIAGNOSIS	MATERIAL	NO OF SPECIMENS TESTED	POLIO	RSSE	UNIDENTIFIED
Paralytic poliomyelitis	Feces	36	22	0	0
Aseptic meningitis	Feces	14	10	0	2
	Blood	22	0	5	1
Encephalitis	Brain	17	1	8	0
	Blood	11	0	1	1

TABLE 152 VIRUSES ISOLATED IN 1956

CLINICAL DIAGNOSIS	MATERIAL	NO OF SPECIMENS TESTED	POLIO	RSSE	UNIDENTIFIED
Paralytic poliomyelitis	Feces	402	62	0	1
	Brain	15	10	1	0
Aseptic meningitis	Feces	457	24	0	1
	Blood	58	0	2	7
Encephalitis	Brain	8	1	1	1
	Blood	7	0	3	1

TABLE 153 POLIO VIRUS TYPES ISOLATED FROM PATIENTS IN 1955 AND 1956

CLINICAL DIAGNOSIS	YEAR	NO OF SPECIMENS TESTED	POLIO MYELITIS VIRUS TYPES		
			1	2	3
Paralytic poliomyelitis	1955	36	17	0	5
	1956	424	67	1	4
Aseptic meningitis	1955	36	8	0	2
	1956	515	19	1	4
Encephalitis	1955	28	1	0	0
	1956	15	1	0	0

Recently the above isolations were repeated using trypsinized human amniotic membrane the results did not improve.

The results of virus isolations are given in Tables 151 and 152 the first showing the numbers of specimens and positive isolates for 1955 when a small number of poliomyelitis cases was recorded and the second for 1956 when an outbreak was observed. Table 153 gives the results

of typing of the strains isolated in 1955 and 1956.

Type 1 polio virus dominated in the epidemic year 1956 as well as in 1955. The percentage of positive isolates of polio viruses is low not surpassing 60 per cent in patients with the diagnosis paralytic poliomyelitis and being just over 10 per cent in patients with the diagnosis aseptic meningitis. Some of the reasons for



the low percentage of virus isolations are as follows

1 The specimens were not collected at the best time so that the virus content of the stool specimens varied and in a number of cases was too low to be detected with the present technic. Some of the tubes inoculated in positive cases remained negative throughout the incubation period in over 20 per cent of the Type 1 positive tests.

2 On many occasions an intermittent excretion of polio virus was observed in 1955 the total isolation rate from feces of paralytic cases amounted to 60 per cent while in 1956 it did not reach 15 per cent but breaking these percentages down considering the individual patient polio virus was isolated in 1955 from 85 per cent of patients with the diagnosis paralytic poliomyelitis and 55 per cent in 1956.

3 The diagnosis of poliomyelitis submitted with the specimen to the laboratory had not been supported in every case by the subsequent clinical course and laboratory data. Even in cases when epidemiologically and clinically a diagnosis of poliomyelitis was indicated the viruses isolated were not always polio viruses. In a case of bulbar paralysis in an ambulance driver who died at the peak of the outbreak an agent was isolated from the brain which was not polio virus and did not belong to the Coxsackie or the ECHO families and was not RSSE mumps herpes simplex or LCM virus.

4 Already during 1953 and 1954 a few strains of Russian spring summer meningo-encephalitis viruses were isolated and identified. In 1955 and 1956 21 strains were isolated which were antigenically similar to if not identical with the viruses of the group of Russian spring summer meningo-encephalitis or louping ill. The findings in 1955 differed greatly from those ob-

tained in 1956. In 1955 nearly 50 per cent of specimens tested were found to be positive for RSSE viruses whereas in 1956 less than 9 per cent of specimens tested were positive for virus. The difference is probably due to the fact that in 1955 the endemic tick borne meningo-encephalitis was prevalent and in 1956 with the poliomyelitis and the ECHO aseptic meningitis outbreak the picture changed and many unsuccessful attempts were made to isolate the virus.

5 Over 10 per cent of all strains isolated and shown in Tables 151 and 152 could not be identified. They were cytopathic agents with variable pathogenicity for white mice the majority being nonpathogenic. We have reasons to believe that a small number of these strains belonged to the Coxsackie family but at least one half to the ECHO viruses as they were isolated mainly in an outbreak of aseptic meningitis which was later proved to be due to ELHO virus Type 9.

The complement fixation test for poliomyelitis was not used in our laboratory and the detection of neutralizing antibodies was in the present state of the clinical diagnosis not very helpful. Nevertheless complement fixation tests for other viruses were performed routinely and found to be valuable on many occasions. The best evidence for this presumption is shown in the results summarized in Table 154.

In 10 per cent of serologically proved cases of RSSE and in 10 per cent of serologically proved cases of mumps the original diagnosis was paralytic poliomyelitis. These percentages are much higher in respect to the diagnosis aseptic meningitis being 46 per cent for RSSE and 62 per cent for mumps virus.

It seems obvious that serologic tests for those viruses known to produce diseases of the central nervous system in the area of the diagnostic

TABLE 154 CORRELATION BETWEEN THE CLINICAL DIAGNOSIS AND THE SEROLOGIC RESULTS

CLINICAL DIAGNOSIS	NO. OF POSITIVE COMPLEMENT FIXATION TESTS FOR				
	INFLUENZA	RSSE	LCM	MUMPS	PTITACOSIS
Influenza	166	4	3	1	1
Pneumonia	46	10	6	11	5
Aseptic meningitis	26	133	25	96	1
Encephalitis	0	91	7	23	0
Poliomyelitis	1	29	1	15	0
Status febrilis	15	31	28	19	2

laboratory are a precious aid in raising the percentage of virologically diagnosed neurotropic illnesses.

Finally I should like to mention how serologically the etiology of a viral infection of the central nervous system can be elucidated. In 1956 we met a form of aseptic meningitis which could not be differentiated clinically from aseptic meningitis caused by the tick-borne RSSE viruses. The laboratory was helpless to diagnose the illness for 10 months in spite of the fact that a few strains were isolated from the blood and the cerebrospinal fluid of some patients. Paired sera were investigated by a number of laboratories for Coxsackie viruses but no definite increase of antibodies could be demonstrated. Through the courtesy of Dr Svedmyr from the Virus Department of the Central Bacteriological Laboratory of Stockholm we obtained a strain of ECHO Type 6 but convalescent sera tested against this virus did not demonstrate neutralizing antibodies. Dr Bossard from the Central Public Health Laboratories Colindale kindly sent us a strain of ECHO virus similar to the Type 9 and named Squirell. In 14 paired sera still left a definite increase of titer of antibodies for the virus could be demonstrated and later over 700 sera of convalescents were collected and showed the presence of specific neutralizing antibodies in high titers in 95 per cent. Even reference laboratories cannot be very helpful on such occasions but international co-operation is needed.

Dr MEENAN: I have two major points for consideration. First in the future there may be a change in the type of diagnostic work which we will be called on to do. Simultaneously with a decline in paralytic poliomyelitis the recognition of the nonparalytic disease and of the symptomless virus excretor will become increasingly important. Unless we examine these intensively we face the danger of driving the virus underground for the paralytic case still represents the

best index of its activity and it will always be essential that we should know what viruses are circulating and at what level in our communities.

The second point is the nature of the specimens which we should use for virus isolation. Using fecal specimens in 1956 we obtained the results shown in Table 155 for nonparalytic cases.

The reason for the relatively high isolation rate is that this was a selected group insofar as all of these cases were children—many from the same county—and that during 1956 which was an epidemic year almost any illness in a child was regarded as poliomyelitis and treated as such until the contrary was proved.

However I feel that if we confine ourselves to fecal specimens alone we may miss a number of virus excretors in the environment of cases at a time when they may be of considerable importance in the spread of infection. In support of this I should like to show the results of studies on one family.

Family No.	Parents and 13 children
10/14	A—ill
10/15	A—clinical poliomyelitis died B—clinical poliomyelitis
10/16	Mouth and throat swabs collected
10/17	C and D—clinical poliomyelitis 8 admitted to hospital for quarantine
10/18	Two more children quarantined One child (O) not living at home not quarantined
10/19	Feces collected
10/20	B died
10/26	E—clinical poliomyelitis

Child	B	C	D	E	F	G	H	I	J	K	L	N	O
Swabs	+	+	+	+	+	+	+	+	+	+	+	+	+
Feces	NS	+	NS	+	+	+	+	+	+	+	+	+	NS

+ = Virus isolated

NS = Not present

In 3 of the 4 cases who had virus present in the throat it was detected also in the saliva.

TABLE 155 RESULTS FOR NONPARALYTIC CASES

CASES	1	2	3	UNTYPED	PER CENT OF ISOLATION
39	2	2	1	4	74

In specimens collected at only 2 points in time poliomyelitis was therefore isolated from 9 members of this family of whom 1 was apparently not at risk and 1 had died before the investigation began. Had feces only and not mouth and throat swabs been examined 2 symptomless members of this family might have been regarded as being free from infection at a time when in fact they might well have been at their most dangerous.

As a result of this and similar although perhaps less spectacular studies I feel strongly that more intensive examination of oropharyngeal specimens would be rewarding in diagnosis and could lead to a reorientation of our thinking on the epidemiology of poliomyelitis—which may have been led astray by the sheer mass of virus in the stools.

PROF. PRZESMYKAJ: The following are some remarks regarding Dr Lennette's report.

1 The percentage of paralytic cases from which the virus could be isolated is somewhat higher in the experience of the State Institute of Hygiene Warsaw than in Dr Lennette's. One should stress the point that 61 per cent of samples have been collected prior to the 10th day of illness and in addition the most material has been provided by one of the Warsaw hospitals. The positive results amounted in paralytic cases to 77.2 per cent and in the nonparalytic cases to 29.9 per cent. 8 (16.6%) strains of polio and 2 orphan strains were isolated from cases with uncertain diagnosis (see Table 156). The material collected was preserved by refrigeration for 2 hours. This table shows how important virologic investigation is for proper diagnosis.

2 In some of the countries in addition to poliomyelitis some other neural infections

chiefly of an encephalitic character are seen. Therefore the number of antigens should be enlarged by addition of the tick borne encephalitis.

3 During the study of neuro-infections quite a number of strains of Coxsackie ECHO tick borne encephalitis and other identified strains are isolated. As yet the etiologic role of these strains is not sufficiently clear. The laboratories meet with great difficulties in the identification of the isolated strains. Standard sera are also lacking.

In order to meet all these needs I move that a center for the study of neuro infections should be established. This institution should work on lines similar to those of the Influenza Center.

Also it is of paramount importance that a course for the training of the virologic laboratory staff prepared for the study of neuro-infections should be organized under the auspices of the WHO.

DR. SMADEL: I have no data to present but I would like to discuss with you the few ideas on where we have been in the diagnosis of polio and some of the other enteric agents and diseases and where we need to go now.

Anyone who attended the meeting in Copenhagen may recall that in presenting a general review of the diagnostic procedures in much the same manner that Dr Lennette presented it this morning I was rather hard put to find enough to talk about in the diagnosis of polio and spent a fair amount of time discussing diagnostic procedures for other related CNS diseases.

I think all of us should be very happy and grateful to the virologist for his having done so well in a few years in developing a series of very precise research tools such as we have heard

TABLE 156. OUTCOME OF TESTS FOR VIRUSES

	EXAMINED	POLIO VIRUS ISOLATED	PER CENT	ORPHAN STRAINS ISOLATED	PERIOD DURING WHICH THE MATERIAL WAS ISOLATED
Paralytic cases	127	98	77.2	4	{ 1-10 = 61.3% 11-20 = 31.8% 21-30 = 6.9%
Nonparalytic cases	61	III	29.9	2	
Cases with uncertain diagnosis	48	8	16.6	2	
Total	236	124	53.4	8	

discussed today I would emphasize though that these are still research tools and that Dr Lennette in presenting his work rightly emphasized the difference between the virus diagnostic laboratory and the virus research laboratory pointing out some of the difficulties which the virus diagnostic laboratory faces. It would be fruitless to quibble about whether the isolation rate is 60 per cent, 70 per cent or 80 per cent. We are really not discussing the value of a particular technique. We are discussing the value of the entire procedure from the time the physician sees the patient on through until the material reaches the sensitive cell. Let us merely be grateful that we have such excellent tools and let us continue to use them.

Let us also ask about the physician who is not close to the very expensive virus diagnostic laboratory which also carries with it research or is not fortunate enough to have a friend who is a researcher in poliomyelitis and is willing to do some of his diagnostic work for him. What about the man who is a long way from these places? He needs some fairly simple tools. They should be reasonably accurate—80 per cent one would settle for. The old Weil-Felix test is a very valuable thing in the rickettsial diseases; it is only about 80 per cent accurate but it is useful. This is the sort of thing we need.

I wonder whether we do not have enough of the technical know-how to begin to get such a test using stool specimens which are about as rich in virus as most of the things we were able to get until a few years ago and using some of the coated particle techniques that we have used very extensively in all sorts of tests in the last few years. I do not know whether or not one would be successful in coating the particles with antibody or in coating them with some of the virus in the stools but I think it is worth a try. One might get a simple quick test by such an approach.

The other thing that we need is a tool to help the epidemiologist—something as good as the typing tool in typhoid fever. There is an indication that such a tool might be at hand also. M. Hilde of the National Institutes of Health in Bethesda reported a technique a few months ago to the American Society of Bacteriologists in which he employed the old phage neutralization technique using the plaques of polio

mixing virus and antisera and diluting these at different time intervals and getting the reduction in plaques with time. This suggests at least the preliminary data suggest that this is a simpler way of telling the difference between strains. Here is another kind of tool that we need. Neither of these tools is ready for use but I think that perhaps before the next Congress we might have them.

DR SVEDMYR: Today's topic has already been very well covered; still I would like to make two minor comments.

I would like to ask Dr Lennette as well as Dr Verlinde whether or not the age distribution among their paralytic patients who were virus positive differed from that of the virus negative ones. During the severe epidemic of poliomyelitis in Stockholm in 1953 in 91 per cent of the cases we recovered Type 1 virus from single stool specimens of paralytic children who were below 16 years of age provided that the specimens were procured within 10 days after onset of the disease. The corresponding frequency for adults was only 68 per cent. A similar age difference was found for the non-paralytic cases of aseptic meningitis; thus 81 per cent of the children but only 49 per cent of the adults were virus positive—again provided that the stools were taken within 10 days after onset. All recovery rates were somewhat lower during the next 10-day period.

It seems probable that the children of 10 at least during our epidemic excreted virus in somewhat larger amounts and probably also for a longer time than did the adults. I would hesitate to speculate about the mechanism of this phenomenon. Possibly it may be correlated to previous experience of polio virus.

I agree with Dr Johnson that for the time being it might be wise not to base an etiologic diagnosis of infection with polio virus or for that matter other enteric viruses on the results of a complement fixation reaction alone. However maybe we should await further clarification of the nature and the frequency of the heterologous reaction which apparently has been encountered in several laboratories before we completely reject the complement fixation test for diagnostic purposes.

PROF DR VERLINDE As I pointed out in the discussion on enteric viruses last Tuesday an epidemic of viral meningitis due to ECHO 9 virus occurred simultaneously in the Netherlands with an epidemic of poliomyelitis. The stool specimens from all over our country of 11 000 000 inhabitants collected by a large number of local physicians without any control of our diagnostic service were sent to our laboratory and examined virologically. Special containers and dry ice were not available for local physicians so that specimens were sent in a variety of bottles by ordinary mail however most of them were sent by special delivery so that the vast majority reached the laboratory within 24 hours.

The specimens were routinely examined by inoculation of monkey kidney cultures and suckling mice.

Because of the simultaneous occurrence of meningitis due to ECHO 9 virus specimens from patients with meningeal involvement were particularly important for the differentiation between meningitic forms of poliomyelitis and viral meningitis of other etiology.

The total number of virus strains isolated was over 1500 originating from 1472 patients which indicates that a virus was recovered from most of them on only one occasion. As a matter of fact more than one stool specimen from the same patient was received only occasionally.

Of these strains over 800 were of the poliomyelitis group and the type distribution was as follows: Type 1 91 per cent, Type 2 8 per cent, Type 3 1 per cent.

Since most of the clinical data which accompanied the specimens were insufficient we are trying to follow up to complete them. So far complete clinical data of 600 patients are now available and on this basis I would compare Dr Lennette's basis with ours.

1 The interval between the indicated onset of illness and the collection of specimens ranged from 0 to 30 days, the median interval being 9 days.

2 In 66 per cent of 200 patients with paralytic poliomyelitis a polio virus was recovered. The viral recovery rate in the first week of illness was over 80 per cent and then in the following weeks decreased gradually. Of 400 patients with the clinical diagnosis of nonparalytic

poliomyelitis—meningeal involvement and abortive cases—only 10 per cent yielded polio virus and from 46 per cent ECHO 9 virus was recovered.

3 In a small number of patients a comparison of the viral recovery rate from stools, rectal swabs, pharyngeal swabs and cerebrospinal fluid could be made. In those who yielded virus from one or more of these specimens the following percentages were found to be positive:

When polio virus was recovered the stools were found to be positive in 75 per cent and the rectal swabs from the same patients in 70 per cent. The pharyngeal swabs gave 21 per cent and cerebrospinal fluids were always negative.

On the contrary when ECHO virus was isolated we found the stools to be positive in 100 per cent, the rectal swabs in only 58 per cent, the pharyngeal swabs in 83 per cent and the cerebrospinal fluid in 28 per cent.

From this small number of patients it appears that the viral recovery rates from rectal swabs and stools do not differ significantly as far as polio virus is concerned. As to the recovery of ECHO virus the viral recovery rate from rectal swabs was inferior to that from stool specimens and even from pharyngeal swabs.

DR VOROSHILOVA Laboratory diagnosis in poliomyelitis is being used more and more widely in the Soviet Union. In the past 2 years in a number of towns special diagnostic laboratories which work under the general methodological guidance of the Poliomyelitis Research Institute were organized. Centralized supply of type-specific immune sera and standard strains of virus is provided for these laboratories.

Isolation of 1140 strains of virus has been described. 4 of these strains have been classified as a strain of Type 4. Let me discuss this question of Type 4 virus. The question of the existence of Type 4 poliomyelitis virus in the USSR arose in 1952 when during an epidemic of poliomyelitis in Karaganda 3 strains of peculiar virus were isolated from stools of patients with paralytic poliomyelitis. 2 of the strains (AB and G7) were isolated by direct inoculation of monkeys. The third strain (MK) was originally isolated in cotton rats and new born white mice and later adapted to monkeys.

Figure 245 presents a diagram of AB strain





FIG 247 *Macacus rhesus* Angara inoculated with AB strain brain suspension from the 26th suckling cotton rat passage (Quadruplegic paralysis of muscles of the back)

litis was carried out through 4 more passages in monkeys. MK strain was isolated from pooled feces of 2 patients with paralytic poliomyelitis by inoculation of adult cotton rats and newborn white mice. As further experiments showed suckling cotton rats were found to be

most susceptible to infection then came adult cotton rats and suckling white mice. Many attempts to inoculate adult white mice always gave negative results.

Thus Karaganda strains differed in their pathogenicity from Type 1 and Type 3 strains, as they caused disease not only in monkeys but also in suckling and adult cotton rats and in suckling white mice. In contrast to Type 1 strains AB, GZ and MK strains are apathogenic for adult white mice. Histologic examination of brain and spinal cord from paralyzed monkeys showed lesions typical for experimental poliomyelitis (Fig. 248 *left*). Besides lesions in the central nervous system myositis was found in suckling white mice and suckling cotton rats (Fig. 248 *right*). A monkey was inoculated with muscle suspension from the 17th suckling cotton rat passage of MK strain. Simultaneously another monkey was inoculated with brain from the same suckling cotton rat. Both inoculated monkeys came down with severe experimental poliomyelitis.

We established the difference of AB and MK strains from Type 1 and Type 3 polio viruses (Figs. 249, 250 and 251) in cross immunity tests in monkeys and we showed the absence of immunologic relationship between AB and MK strains on the one hand and Type 1, Lansing and OVCH strains on the other (Figs. 252 and 253) in experiments on cotton rats.

On the basis of these facts and considering the absence of immunologic relationship with

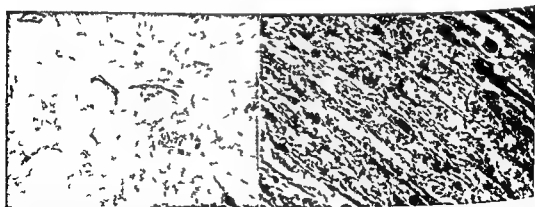


FIG 248 (*Left*) Photomicrograph showing massive degeneration of nerve cells in lumbar anterior horn of *Macacus rhesus* Lastic inoculated with stools of patients Achundova and Belik AB strain. (*Right*) Diffuse myositis in a suckling cotton rat inoculated with AB strain.

## ОПЫТ ПЕРЕКРЕСТНОГО ИМУНИТЕТА

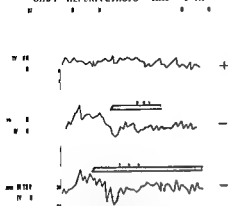
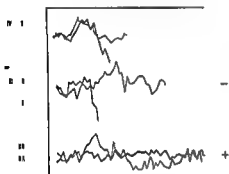
ОПЫТ ПЕРЕКРЕСТНОГО ИМУНИТЕТА  
ЗАРАЖЕНИЕ ШТАММОМ ИВ ОБЪЕЗЫН ИМУНИЗМЫХ  
К IV I H I ТИПУ ВИРУСА ПОЛИОМИЕЛАНТА

Fig. 249 (Top) Cross immunity test monkeys immune to Type 1 and Type 3 polioviruses are infected with AB strain

Fig. 250 (Bottom) Cross immunity test monkeys immune to Type 1, Type 3 and Type 4 polioviruses are infected with Type 1 TV strain

Type 1, 2 and 3 strains of polioviruses a suggestion was made that these strains from Kari-ganda should represent the fourth immunologic type of poliovirus.

At the same time some peculiarities of the above strains were noted. In histologic examination of a large number of monkeys in about 50 per cent of cases besides marked poliomyelitis lesions in the spinal cord and the medulla some small inflammatory foci in

## ОПЫТ ПЕРЕКРЕСТНОГО ИМУНИТЕТА

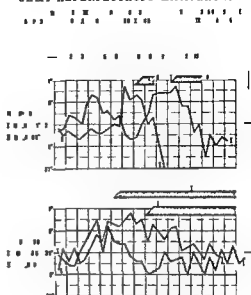


Fig. 251 Cross immunity test monkeys immune to Type 1, Type 3 and Type 4 polioviruses are infected with Type 1 Kari-ganda and Type AB infection are inoculated with Type 3 Leon virus

frontal temporal parietal and occipital lobes of the cerebrum were found also there were more extensive lesions in the medulla than were observed in Type 1 poliovirus infection. AB and Mh strains produced myositis in suckling cotton rats and suckling white mice experimental poliomyelitis has been reproduced by inoculation of monkeys with muscles from these paralyzed sucklings.

In 1957 Type 4 AB strain was sent for study to the Karolinska Institute in Stockholm to the Pasteur Institute in Paris and to several laboratories in the U.S.A. According to Dr. K. Habel and Dr. L. Loomis and also Drs. D. Horstmann and E. Monod's our fundamental data on the properties of AB strain were confirmed in these laboratories.

In 1956 Doctor Johansson (Stockholm) found antigenic similarity between AB strain and Coxsacke A7 strain. This similarity was confirmed serologically in our laboratory too when we got all the 74 types of Coxsacke viruses from Czechoslovakia.



ПЕРЕКРЕСТНЫЙ ИММУНИТЕТ У ХАПОКОВЫХ КРЫС  
ИММУНИЗИРОВАННЫХ ШТАММАМИ ВИРУСА ТИПОВ II И IV

Таблица 221 Иммунизация и тестирование	Титры антител к вирусам типов II и IV			
	IV AB	IV MK	II OVЧ	II Антисит
IV AB	>10000	>1000	25	<100
IV MK		>316		0
IV ГЗ		162		12
II OVЧ	12	14	100000	10000
II Антисит	10	0	100000	10000

ОПЫТЫ НЕЙТРАЛИЗАЦИИ НА ХАПОКОВЫХ КРЫСАХ  
ШТАММОВ ТИПА II И IV ИММУНЫМИ АНТИСЫВОРОТКАМИ

Экспериментальные антисыворотки к штаммам	Титры антител к вирусам			
	IV AB	IV MK	II OVЧ	II Антисит
IV AB	>4		0.67	0
IV MK		>34		<14
II OVЧ	0		333	30
II Антисит	0	<14	>333	

FIG 252 (Top) Cross immunity in cotton rats immunized with Type 2 and Type 4 viruses

FIG 253 (Bottom) Cross neutralization test in cotton rats with Type 2 and Type 4 strains

Together with Golubeva we inoculated monkeys and adult cotton rats with Coxsackie A7 virus and the first monkey inoculated with muscle suspension from paralyzed suckling white mice developed fever paresis of lower extremities and typical histologic poliomyelitic changes in the spinal cord (Fig 254). Coxsackie A7 strain was pathogenic also for adult cotton rats and regularly produced paralyzed animals (Fig 255).

Thus the characteristics of Coxsackie A7 strain as well as the characteristics of Karaganda AB-4 GZ-4 and MK-4 strains do not correspond to the definition of the Coxsackie group of viruses because of marked pathogenicity for monkeys and cotton rats and capacity to produce poliomyelitislike lesions in the central nervous system of these animals.



FIG 254 Photomicrograph showing appearance of the inner group of cells in the lumbar cord of macacus rhesus inoculated with Coxsackie A7 virus



FIG 255 Coxsackie A7 virus Paralysis of both hind legs in cotton rat 9th day after infection

The capacity to produce myositis in monkeys was established in our Institute not only for strains of Coxsackie A7 AB-4 but also for one strain of Type 2 polio virus also immunologically distinct from the strain AB-4. The Karaganda strains possess marked neurotropism in monkey experiments consequently one cannot be sure that they will not cause poliomyelitis in human beings. Direct isolation in monkeys of virus from patients who died of the bulbar spinal form of poliomyelitis in Karaganda also shows the necessity of new studies directed toward elucidation of the etiologic role of this group of viruses in human disease.

Doctor Sven Gard wrote in 1955: "The Coxsackie group is apparently not homogeneous it is very probable that some of its present members will be classified differently in the future."

We are inclined to think that the data on Coxsackie A7 strain and AB-4 strain accumulated in the 4 laboratories in different countries

## Discussion

of the world are contrary to the main character-  
istics of the Coxsackie group of viruses and  
they rather confirm the opinion of the possibility  
of grouping them into an independent Type 4  
polomyelitis virus or a special virus of para-  
polomyelitis infection. Then we have Types 1  
and 3 polomyelitis viruses which are strictly pathogenic  
for monkeys. Type 2 which is pathogenic for  
monkeys while mice and occasionally may in-

duce myositis in sucklings then Type 4 Cox-  
sackie A7 which is pathogenic for monkeys for  
suckling cotton rats and sucklings and then  
Coxsackie groups of viruses themselves. Further  
investigations should be carried out on the  
prevalence of viruses of this group and also  
more complete studies should be made on patho-  
genicity spectrum of other members of Coxsackie  
and ECHO groups of viruses.



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# Basic Problems of Respiratory Distress in Patients with Poliomyelitis

WEDNESDAY MORNING, JULY 10, 1957

(This Session Convened in the Aula of the University of Geneva)

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## *Chairman*

DR PIERRE RECHT  
European Association  
Against Poliomyelitis  
Iussels

## *Speakers*

DR J H COMROE JR  
University of Pennsylvania  
Medical School  
Philadelphia

DR JERE MEAD  
Harvard University  
School of Public Health  
Boston

DR JAMES I WHITTENBERGER  
Harvard University  
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DR JAMES F BOSMA  
University of Utah  
College of Medicine  
Salt Lake City

DR CLARENCE W DAIL  
Rancho Los Amigos Hospital  
Hondo California

## *Discussants*

DR C M ARDFAN  
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Nuffield Institute for Medical Research  
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DR H HEEMSTRA  
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Physiological Institute  
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DR CLARENCE P COLLIER  
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DR HELMUT M KIRSCHSIEPER  
Universitäts Kinderklinik  
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PROF THIÉPHE MOLLAPET  
Hospital Claude Bernard  
Clinic of Infectious Diseases  
Paris

# Assessment of Respiratory and Pulmonary Function in Patients with Poliomyelitis

DR J H COMROE, JR

The ideal management of a patient with respiratory failure either acute or chronic requires a physician who has an expert knowledge of respiratory physiology and of pulmonary function.

Although the function of the lungs is a simple one—that of pulmonary gas exchange—this function is achieved by a series of processes.

Figure 256 top left shows the first of these processes namely that of ventilation and the most important effect here is that there shall be an adequate volume of fresh air entering the respiratory passages the conducting airway down into the small air sacs or alveoli of the lungs where rapid exchange of oxygen and carbon dioxide occurs.

Figure 256 top right shows that in addition to the volume of ventilation it is important that there be even distribution of the inspired air so that the air entering the nose and the mouth is distributed equally to all of the millions of alveoli or air sacs in the lung.

Figure 256 bottom left indicates the third of these processes namely that of diffusion. After air enters the airway and is distributed evenly to the alveoli it must diffuse across the alveolar capillary membrane into the pulmonary capillary blood.

Figure 256 bottom right shows the last of these processes. Here one has mixed venous blood entering the pulmonary capillaries coming into contact with alveolar gas becoming arterialized and oxygenated very quickly and then leaving as arterial blood. It is important that pulmonary blood flow be adequate in volume and also even in its distribution to all the alveoli in the lungs.

In addition you see that these processes do require work. Work is required on the part of the respiratory muscles to accomplish ventilation. Work is required on the part of the right ventricle to push capillary blood through the

pulmonary capillaries. Therefore pulmonary function is normal only when ventilation diffusion and blood flow are normal and when the work required to accomplish these is also normal. These facts are simple these facts are elementary everyone knows them and almost everyone forgets them. Therefore I would like to remind you that it is not enough to measure the rate the depth or even the minute volume of respiration and on the basis of this call ventilation normal or abnormal.

Figure 257 shows 3 different combinations of alveolar ventilation. B is the record of an individual breathing normally 500 cc with 16 breaths a minute minute volume 8000 ml. A is an individual breathing twice as fast and half as deeply 32 times per minute 250 ml per breath and again he has the same minute volume 8000 ml. C is a subject breathing very slowly and deeply 8 times a minute 1000 ml per breath again a minute volume of 8000 ml. In each case the minute volume is the same but the useful ventilation that is the alveolar ventilation is different in each. In A it is 3,200 ml which is too little in B it is 5,600 which is about right in C it is 6,800 which is too much and results in hyperventilation.

Now the reason for these differences in useful or alveolar ventilation is because of the respiratory dead space. It is possible to measure the anatomic respiratory dead space.

Figure 258 shows one way of measuring the anatomic dead space. This is an individual breathing air. He takes one deep breath of pure oxygen and at the indicated point breathes out. The air passes through a nitrogen meter which records instantaneously and continuously nitrogen concentration in the expired air. The final plateau represents alveolar gas that has been expired later I will mention that this is important in evaluating how evenly air is distributed among the alveoli. A represents the washout

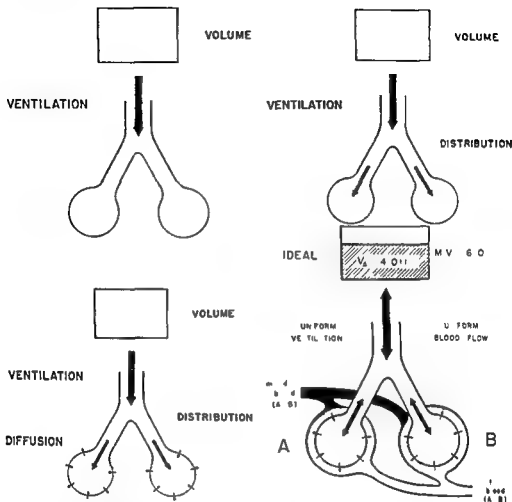
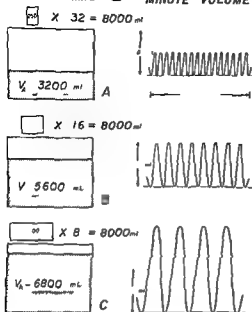


FIG 256 (Top left) Ventilation process showing volume of air entering respiratory passages (Top right) Even distribution of air entering respiratory passages (Bottom left) Diffusion of air into the pulmonary capillary blood (bottom right) Changing of venous blood to arterial blood in the pulmonary capillaries

of the dead space analysis of this enables one to calculate and measure the anatomic dead space. However, I would like to point out that even though one knows accurately this anatomic dead space, one still does not have all the information that one needs to evaluate ventilation and pulmonary function. The reason is that the amount of ventilation that an individual needs must be related to his activity at that moment—his oxygen consumption, his  $\text{CO}_2$  production, and this depends on the degree of his muscular

activity, whether or not he has fever, and other factors. It is also important to remember that even though the minute volume, the dead space, and the rate are normal in relation to the amount of activity of the patient, there still may be anoxemia; there still may be  $\text{CO}_2$  retention if the ventilation of the patient is uneven, if the pulmonary capillary blood flow is uneven, or if the diffusion across the alveolar capillary membrane is impaired. Therefore, it is important in some cases to know not only the volume of ven

TIDAL VOLUME  $\times$  RATE = MINUTE VOLUME



(TV DS) RATE ALVEOLAR VENTILATION ( $V_A$ )

FIG 237 Different combinations of alveolar ventilation

tilation but also whether it is uniform or not and the single breath of oxygen using the nitrogen meter as shown in Figure 258 is a good quick test of the uniformity of the alveolar ventilation

It is also important to think in terms of the

diffusing capacity of the lungs. It is now possible to measure quickly and easily the diffusing capacity of a patient's lungs. This is a test using carbon monoxide according to the old technique of Marie Krogh somewhat modified by Dr Forster in our department.

A patient who is breathing air normally expires and then breathes in deeply a mixture of low nontoxic concentration of carbon monoxide and some helium as a tracer gas. The individual holds his breath for 10 seconds, then blows out. The alveolar gas is collected and from this one can make calculations of the diffusing capacity of the lungs. One can make these measurements quickly and one can repeat them frequently; they involve no blood samples, no cardiac catheterization. From such data one can have an idea of the area of the ventilated alveoli in the lungs that are in contact with pulmonary capillary circulation and one can also have an idea of the thickness of the alveolar capillary membrane.

One might ask is it ever necessary to know anything in the face of poliomyelitis except the volume of ventilation? The answer is yes; it is necessary. Uneven ventilation may occur owing to inhalation of secretions, localized infection in the lung, localized edema in the lung, or even pneumothorax. Impaired diffusion may occur because of pulmonary edema, either in the tissues of the lung or the alveoli. Impaired diffusion

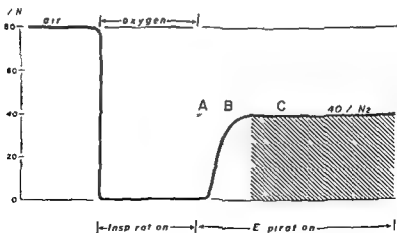


FIG 258 Single breath analysis (nitrogen meter)

sion may occur because of inflammation or pneumonia. Uneven blood flow may occur because of atelectasis, collapse of the lungs, or pulmonary embolism or thrombosis.

Are there any simple over-all tests of pulmonary functions that help to evaluate the patient? In order to do this one must know the arterial oxygen and the arterial carbon dioxide. The arterial-oxygen saturation may be measured by an oximeter and is of particular value when one asks the individual to breathe 100 per cent oxygen for about 10 minutes after breathing room air to see how high the arterial-oxygen saturation then rises. It may be possible within a year to measure the oxygen tension or pressure in the arterial blood and to do this much more rapidly than to measure arterial-oxygen saturation. The oxygen electrode has been greatly improved and may come into widespread use fairly soon. The arterial carbon dioxide pressure or tension may be measured without sampling arterial blood if one obtains a sample of alveolar gas after the dead space has been washed out. This  $\text{CO}_2$  may be measured either with an infrared analyzer or by very simple chemical techniques using the Scholander 5 ml analyzer and an ordinary 5 ml syringe. I believe that in the complete evaluation it is not



Fig 259 Plethysmograph or body box for testing pulmonary function

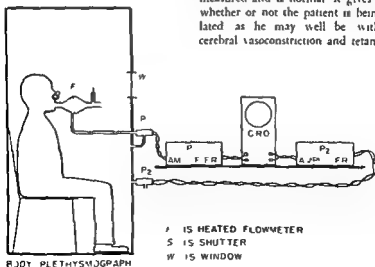


Fig 260 Schematic drawing of plethysmograph

enough to measure carbon dioxide alone or to measure oxygen alone. If the oxygen alone is measured and is normal, it gives no clue as to whether or not the patient is being hyperventilated as he may well be with consequent cerebral vasoconstriction and tetany. If the car-



bon dioxide alone is measured and is found to be normal or even low this gives no clue as to an impairment of diffusion because in such cases the  $\text{CO}_2$  may be normal or low

There is a new method for testing some aspects of pulmonary function using a body box or a body plethysmograph

Figure 259 shows an individual sitting within the body box a box about as big as a telephone booth The individual sits within the box and breathes air from around him He does not become anoxic because he is there for only a few minutes and there are 600 liters of air in the box The door is then closed and pressures are measured within the box

Figure 260 shows a scheme of the individual within the box breathing through a flowmeter While he is breathing the pressure within the box is measured continuously by a very sensitive electrical manometer ( $P$ ) and the pressure in the airway is measured by another sensitive manometer ( $P_1$ ) Flow and pressure are measured simultaneously and recorded on the cathode ray oscilloscope By means of this body box and a number of different techniques we have now measured the thoracic gas volume the volume of trapped air within the lungs pulmonary capillary blood flow airway resistance and pulmonary tissue resistance

Figure 261 illustrates the principle involved in one of these measurements that is of measuring airway resistance To measure airway resistance one must know alveolar pressure and

flow Flow is measured by a pneumotachograph Alveolar pressure is measured in the body box When the individual breathes in during inspiration the air in the lungs is at a lower pressure than than atmospheric This air is expanded and this compresses the air in the box so that the gauge shows an increased reading By taking measurements of this during inspiration and expiration one can measure alveolar gas pressure continuously during flow and so measure airway resistance

A large number of pulmonary tests can now be done on patients One can measure the volume of ventilation One can measure its distribution One can measure diffusion One can measure pulmonary capillary blood flow So far as mechanical tests are concerned one can now measure the resistance in the airway the resistance in the pulmonary tissues and the compliance But Dr Mead and Dr Whittenberger who follow me will talk to you more about these measures These new tests of pulmonary function have now been applied to patients who have recovered from poliomyelitis Approximately  $\frac{1}{4}$  of the patients who have had poliomyelitis and respiratory muscle paralysis when they recover developed clinically detectable scoliosis Many surgeons have tried to correct these deformities of the thorax and of the spine Often they may be very severe deformities However there has been no systematic attempt to measure pulmonary function in individuals

$$\text{AIRWAY RESISTANCE} = \frac{\text{ALVEOLAR PRESSURE}}{\text{FLOW}}$$

expir

during inspiration

during expiration

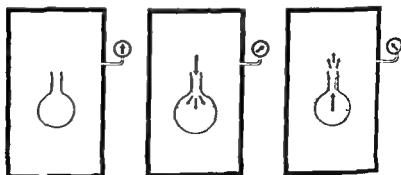


FIG 261 Principle of measuring airway resistance

who have recovered from poliomyelitis and who now have thoracic or spinal deformities. Studies are now under way in our department by Dr. Thomas Gucker, an orthopedic surgeon who has a deep interest in problems of pulmonary physiology. This study will take several years in order to follow these individuals as they are treated by long term orthopedic procedures. Twenty two patients have been studied so far. In a number of these patients the vital capacity is greatly reduced, the maximum breathing capacity is greatly reduced, the flow rates may be very low indeed. The compliance of the lungs is often much reduced. The airway resistance measured in the body box is often greatly increased. Therefore you see that before any orthopedic procedures are attempted these patients have severe impairment of the function of the lungs and the thorax. It is important to

find out whether or not the standard orthopedic procedures make these conditions worse. In some instances, particularly those involved in extensive immobilization by the surgeon, pulmonary function has been made much worse during the period of treatment.

In the management of poliomyelitis, remember that the respiratory difficulties of the patient are not necessarily at an end. Even when the patient has recovered from the acute attack and has gone home, he may within several years develop scoliosis and severe respiratory impairment on this account. Surgeons or other physicians who engage in corrective procedures of the spine and thorax of these patients should consider seriously the pulmonary and respiratory difficulties and make objective tests of pulmonary functions before, during and after the orthopedic procedures.

# Principles of Respiratory Mechanics and Coughing

DR JAMES L WHITTENBERGER

The mechanics of breathing have been little taught in medical schools until the past 5 years. This aspect of respiratory physiology is basic to understanding artificial respiration and pressure breathing.

In breathing the respiratory muscles must move the thorax and the abdomen, the lungs and the gas in the passages within the lungs and the respiratory tract. In accomplishing this movement two principal properties of the system are encountered. These are *elastic* and *flow resistive* properties of the lungs and the thorax. The flow resistive properties include the viscous resistance offered by the pulmonary and the thoracic tissues and the gas flow resistance offered by the air passages.

We will consider first the elastic properties exhibited by the lungs and the thorax. The elastic recoil of the chest normally tends to expand the lungs and the elastic recoil of the lungs tends to collapse them. If the respiratory muscles are entirely relaxed or paralyzed by a relaxing drug or poison these opposing elastic forces are in equilibrium at a level of lung volume which may be called the *resting end expiratory volume* or the *functional residual capacity*. In the upright posture the intrapleural pressure at this volume is approximately 5 cm of water less than atmospheric pressure. In the supine position the weight of the abdominal contents pushes the diaphragm in the expiratory direction and the elastic equilibrium between the lungs and the thorax is shifted to a smaller volume. Since the lung volume is smaller the elastic recoil is less and the resting end-expiratory intrapleural pressure is closer to atmospheric—perhaps 3 cm of water less than atmospheric.

During inspiration the respiratory muscles increase the volume of the thorax and the lungs above the equilibrium volume. Thereby the lungs are stretched further and their force of elastic recoil is increased. This increased force is reflected in a fall in intrapleural pressure that the intrapleural pressure becomes increasingly

subatmospheric as lung volume increases. At the end of inspiration the inspiratory muscles relax and the lungs and the chest wall are forced back to the equilibrium volume mainly by the elastic recoil of the lungs. If expiration continues further into the expiratory reserve compartment the expiratory muscles are increasingly called on to deform the thorax.

The respiratory muscles encounter opposition of another sort. They must supply the force necessary to produce flow of gas through the upper airway and the tracheobronchial tree and additional force to produce what might be called flow of the lung parenchyma and the moving tissues of the thoracic wall. During quiet breathing the respiratory muscles need supply these forces only during inspiration; the elastic recoil of the lungs is sufficient to overcome gas and tissue resistance during ordinary expiration.

Just as the force required to oppose the elastic recoil of the lungs is reflected in the intrapleural pressure, so are the forces necessary to overcome gas and tissue resistance of the lungs and airways. For example, during a rapid inspiration the expanding chest wall may be visualized as pulling on the lung surfaces and the lungs resist the pull not only with their own elastic recoil but also with the flow resistance of the airways and the moving tissue. As a result, intrapleural pressure drops further below atmospheric pressure than can be accounted for by the elastic recoil alone. During a forced rapid expiration the elastic recoil of the lungs is insufficient to produce so rapid a volume change. Due to the action of expiratory muscles the chest wall may be thought of as pushing on the pleural surface of the lungs and the intrapleural pressure may be driven well above atmospheric pressure.

Thus the pressure fluctuations at the surface of the lungs are not simply a result of lung elasticity—they relate also to flow resistance. This is true during quiet breathing as well as during forced breathing. If we are to obtain

a measure of the elastic and flow resistive properties of the lungs we must have a means of separating the intrapleural pressure fluctuations into elastic and flow resistive components. An understanding of this process is the most important step toward understanding the principles of respiratory mechanics.

The features of this analysis may be stated as follows:

1 The elastic component of intrapleural pressure depends on the degree of stretch of the lungs and hence on the volume of the lungs. It is independent of the rate at which lung volume is changing.

2 The flow resistive component of intrapleural pressure depends on the rate at which lung volume is changing and is essentially independent of lung volume per se.

At two points during the respiratory cycle, namely at the end of inspiration and the end of expiration, there is no flow in the normal lung. At these instants intrapleural pressure depends entirely on elastic forces. In the interval between these instants the lungs have changed volume. If we relate this volume change to the associated change of intrapleural pressure measured at the instants of no flow, we obtain a quantitative description of the elastic behavior of the lungs over this range of volume. Expressed as volume change per unit of pressure change (for example in liters/cm of water) the result is termed the *pulmonary compliance*. Expressed as the change in pressure per unit of volume change, this relationship has been called the *elastic resistance* or the *elastance* of the lungs.

Before we consider measurement of flow resistance it is useful to examine the adequacy of the measurement of pulmonary compliance as a description of the elastic properties of the lungs. So far we have related *changes* in lung volume to changes in intrapleural pressure, saying nothing about the absolute levels of lung volume or pressure difference between the air passages and the lung surfaces. For a more complete description of the elastic behavior of the lungs it is necessary to know the relationship between lung volume and the elastic component of intrapleural pressure over a wide range of lung volume from complete collapse to maximal expansion. Further it would be desirable to take into account the size of the lungs

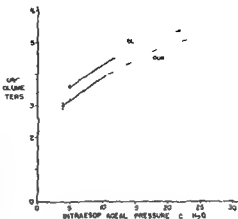


FIG. 767 Graph showing relationships of lung volume and the elastic component of intrapleural pressure.

that is the mass of lung tissue exclusive of the gas contained. The importance of this factor may be illustrated by comparing compliance measurements made in two animal species of different body size for example the guinea pig and man. The pulmonary compliance of an average man is 180 ml/cm of water. The pulmonary compliance of small guinea pigs is 0.7 ml/cm of water, roughly one thousandth that of man. However when lung size is taken into account by expressing compliance per gram of lung tissue, the lungs of the guinea pig and of man are found to be closely similar in their elastic behavior.

It is not possible in practical measurements made during life to obtain accurate information about lung tissue mass or to study the elastic behavior in terms of absolute volume or pressure over a full range of lung expansion. However it is possible to approach these ideals. As an index of lung size we may take a measure of body size for example the height. It is reassuring to note that measurements of pulmonary compliance made in infants, children and adults vary approximately in the same ratio to body height as does lung weight.

The lung volume change can be extended much beyond the resting tidal range voluntarily or by means of externally applied pressure as in a respirator. Further the absolute volume of gas in the lungs can be measured by gas dilu-

tion methods or more conveniently by the plethysmographic methods similar to those that have recently been developed in Dr. Comroe's laboratory. It is the problem of measuring absolute intrapleural pressure safely and accurately that at present stands in the way of the ideal description of the elastic behavior in terms of absolute volume and pressure. The measurement of intra-esophageal pressure has provided a safe and convenient approximation of intrapleural pressure change as demonstrated by direct comparison of esophageal with intrapleural pressure. However as yet the validity of esophageal pressure as a measure of absolute level of intrapleural pressure has not been established. It appears that it offers a useful approximation at least in subjects studied in the upright position.

An example of the usefulness of placing the relationships of lung volume and the elastic component of intrapleural pressure on an absolute scale is illustrated in Figure 262. The volume of gas in the lung is plotted on the ordinate against the intra-esophageal pressure relative to atmospheric pressure. The two solid lines represent mean values obtained for a group of young subjects and a group of elderly healthy human subjects, the subjects being matched for body height. These lines represent the measurements made in the tidal range of lung volume. The slopes of the lines are equivalent to compliance values that would be obtained during quiet breathing. The slopes of the lines are nearly the same for the two groups, the young and the old, and indeed the difference in compliance is not statistically significant. One might conclude from this much information that the lungs of elderly individuals behaved elastically in the same way as the lungs of young subjects. This is not the case as may be seen when we compare the volumes and the pressures in absolute terms. The functional residual capacities of the two groups of subjects were measured by a gas dilution method and all volume changes related to this volume. When we compare the transpulmonary pressure at equal lung volumes we see that in all volumes the pressure is less in the older subjects. Therefore we may conclude that in general the lungs of older individuals demonstrate a diminished elastic recoil compared with the lungs of younger individuals. This

finding is consistent with the observation that the functional residual capacity increases slightly with age.

When we consider the measurement of flow resistive properties, it is important to note the shape of the elastic characteristics in the ordinary range of breathing. In this range of volume a straight line relationship exists between the volume and the elastic component of transpulmonary pressure. This fact is of use in isolating the flow resistive component of pressure for it may be seen that if we know the compliance of the lungs we can predict the elastic component pressure at any point during the respiratory cycle. All we need to know is how much the volume has changed from the no flow point at either extreme of tidal volume. Having predicted the elastic component at any instant during the respiratory cycle we can compare this pressure with the observed pressure at the same instant and the difference will represent the pressure overcoming flow resistance offered by the air passages and moving tissues.

Knowledge of the principles of respiratory mechanics helps one to understand the pathologic physiology of respiratory poliomyelitis and the treatment of respiratory paralysis by any form of artificial respiration. In the time remaining I shall be able to deal with only one aspect of respiratory mechanics, namely the physical characteristics of a natural cough and attempts to simulate these events by mechanical devices.

A natural cough (Fig. 263) utilizes the respiratory muscles in such manner that a violent blast of air is forced out through a greatly narrowed tracheobronchial tree. The mechanical advantage of the expiratory muscles is enhanced by a preliminary inspiratory effort. While the glottis is closed expiratory effort begins and intrathoracic pressures build up to 80 or 100 ml of mercury. The resistive pressure drop is indicated on the abscissa and the rate of flow in liters per minute on the ordinate. As one begins to cough he takes in an inspiration, then the pressure in the thorax is filled up to 80 or 100 ml of mercury, sometimes even higher, before the glottis is opened. Since no volume change occurs in this phase except by simple compression



that the compression of the tracheobronchial tree was as important to the objective of the cough as the generation of high flow rates.

Several machines have been developed to imitate a cough in patients with weakened respiratory muscles. These machines are to a variable extent successful in generating a high expiratory flow rate. Figure 264 shows first a normal cough in which the initial pressure was over 120 mm Hg of mercury the pattern of resist-

ance change which relates to the degree of compression of the tracheobronchial tree and the curve for one of the cough simulating devices available in the United States. It is obvious that these curves are very different. The apparatuses, in general, do not generate a high resistance in the lungs and one may infer that there is little or no compression of the trachea. Therefore, such machines do not imitate one of the important characteristics of a cough.

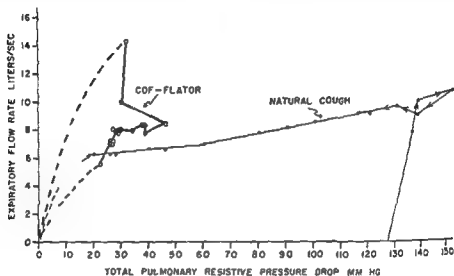


Fig 264 Illustration of natural cough and imitative cough generated by a cough machine

## *Evaluation of Respiratory Mechanics in Patients with Respiratory Muscle Paralysis*

DR JERE MEAD

Dr Whittenberger has presented the principles of respiratory mechanics and has applied these principles in explaining the cough mechanism. I should like to extend the application of these principles to the evaluation of the respiratory mechanical status of patients with respiratory muscle paralysis.

In the patient with respiratory muscle paralysis respiration is accomplished by means of devices which apply pressure to the respiratory system. It is a simple matter to measure the amplitude of the pressures applied, the number of pressure cycles per minute and the ventilation accomplished. This much information will suffice to permit useful deduction as to the mechanical properties of the patient's respiratory system.

Dr Whittenberger has said that two physical properties govern the relationships between applied pressure and ventilation. These are first the elastic recoil of the lungs and the chest wall and second the flow resistance offered by the air passages and the moving tissue. It will simplify matters if we take up these two physical properties separately.

If all we had to consider were the elastic properties of the lungs and the chest wall we could state quite simply the relationship between the frequency and the amplitude of applied pressure and the minute ventilation of the lungs. In such a case the tidal volume would be directly proportional to the pressure amplitude within fairly broad limits. Doubling the amplitude of pressure from 8 cm H<sub>2</sub>O to 16 cm H<sub>2</sub>O would nearly double the tidal volume. Redoubling the pressure amplitude from 16 to 32 cm H<sub>2</sub>O in general would not redouble the tidal volume; at such large tidal volumes the elastic limits of the lungs and the chest wall would be approached and the compliance would be effectively reduced. However, in general tidal volume would be proportional

to pressure amplitude in a purely elastic system. Furthermore, in a purely elastic system tidal volume would be independent of the cycling rate itself. It would not matter how rapidly the pressure changed. As long as flow resistance were absent the volume would be in phase with pressure and at a fixed amplitude of pressure tidal volume would not decrease as the cycling frequency increased. Thus in a purely elastic system the tidal volume is directly proportional to the amplitude of applied pressure and is independent of the cycling rate.

The relationship between applied pressure amplitude and cycling rate and the resulting tidal volume is not as simple as in the purely elastic system. In this instance we will find it useful to concentrate on the minute ventilation that is the product of the tidal volume and the cycling rate rather than on the tidal volume itself. The minute ventilation expresses the average rate of flow of gas for example in liters per minute passing into and out of the lungs. The average rate of flow or minute ventilation is the same in either direction. This would be assuming equal timing for the two phases of respiration for simplicity. In a pure flow resistance system the minute ventilation depends on the average driving pressure in either direction. The average driving pressure is related to the amplitude of pressure. Thus we would predict that in a pure flow resistive system the minute ventilation will vary directly with the amplitude of applied pressure. What can we say about the influence of the cycling rate? The average driving pressure during either phase of respiration will be the same for a given amplitude of pressure no matter how frequently the pressure is cycled. Therefore minute ventilation will be independent of the cycling rate in this hypothetical case. If this is so the tidal volume must decrease as the cycling rate is increased since the minute ventilation is



the product of the cycling rate and the tidal volume

We can now summarize our consideration of these two hypothetical cases. In a purely elastic system tidal volume is proportional to the amplitude of pressure and remains the same as the cycling rate is increased. In a flow resistive system tidal volume is proportional to the amplitude of pressure but decreases as the cycling rate is increased. In the first case at a fixed amplitude of pressure minute ventilation will increase in proportion to the cycling rate. In the second case minute ventilation will remain the same as the cycling rate is increased.

If we now apply to our hypothetical systems the concept of *effective* or *alveolar* ventilation as developed by Dr. Comroe in his presentation it becomes apparent that increases in cycling frequency at a fixed pressure amplitude will lead to a progressive reduction in *effective* ventilation if the impedance to ventilation is primarily flow resistive. For if the minute ventilation is nearly constant and hence tidal volume inversely proportional to cycling frequency the dead space which represents a more or less fixed and ineffective portion of each tidal volume will receive an increasing proportion of the total ventilation as frequency is increased. The remaining effective part the alveolar ventilation will be reduced accordingly.

On the other hand if the impedance to ventilation is primarily elastic since the tidal volume will be maintained as the cycling rate is increased the effective portion of each tidal volume will tend to remain the same and effective ventilation will increase as the cycling rate is increased.

Now let us apply these considerations to real rather than hypothetical examples. The patient lies somewhere between the extremes we have examined. He may approach one or the other of these extremes if he develops intercurrent pathology in his lungs or chest wall such as Dr. Comroe and Dr. Whittenberger have discussed. We can now employ the relationships between frequency and tidal volume to obtain information as to the nature of his difficulty and to help us predict the most efficient pattern of pressure with which to assist ventilation.

The equipment necessary is quite simple. We will need some means of recording ventilation

for example a low resistance spirometer or a low resistance valve system and a gas collection bag. I emphasize the importance of low resistance in the recording equipment because the respirator must supply the pressures necessary to move gas through the measuring devices as well as gas in and out of the patient. If these pressures are appreciable the result that we obtain will be more a measure of the mechanical properties of the measuring equipment than it will be of the patient. The simplest means of measurement consists of a low resistance valve system and a gas collection bag. If the resistance of the valve and bag system is less than 1 cm H<sub>2</sub>O for a flow of 1 liter per second it will be adequate for our needs by and large.

The range of adjustment of pressure amplitude and cycling rate for the particular respirator in use is also of importance to us in these measurements. Fortunately the requirements are not severe. If we can make a twofold change in cycling rate and at the same time adjust the pressure amplitude so that it is approximately equal at the two frequencies our present needs will be met and this is within the range of adjustment of most apparatus.

Let us measure the tidal volume at two frequencies for example at 10 cycles per minute and 20 cycles per minute taking care to maintain the pressure amplitude the same. If the tidal volume is reduced only slightly we can be sure that the principal mechanical impedance to ventilation is elastic in nature. On the other hand if we find that the tidal volume is nearly cut in half when the frequency is doubled we can conclude that the mechanical impedance is principally flow resistive. If we have made some measurements prior to the development of intercurrent pathology and know how much tidal volume decreases when cycling rate is doubled at the same pressure amplitude we can interpret changes in these measurements in terms of underlying mechanical abnormalities. For example complete obstruction of a main bronchus will result in a substantial reduction in compliance. Gastric distention will also reduce compliance. Such patients would show little change in tidal volume with increased cycling rates. On the other hand the increased flow resistance resulting from partial obstruction of the airway or from bronchoconstriction would result in a pro-

gressive reduction of tidal volume with increased cycling rate

Now that we have developed simple means of testing whether the mechanical impedance to ventilation is primarily elastic or flow resistive we can consider the factors determining the optimal pattern of applied pressure to be used in a given circumstance. We will consider the several attributes of the pressure pattern separately. In each instance we will assume that the other attributes of the pattern are held constant. We will first take up the implication of the rate of cycling. Later we will consider the amplitude of pressure, the mean level of pressure, the shape of the pressure pattern and the timing of the phases of respiration.

The influence of the rate of cycling per se is implicit in the material that has been covered. If the principal mechanical impedance to ventilation is elastic then since tidal volume tends to be maintained at the same level as the frequency of cycling is increased effective ventilation will increase more or less in direct proportion to the rate of cycling. If the principal impedance is flow resistive tidal volume will be inversely proportional to the rate of cycling, and although total ventilation will be little affected the effective portion of ventilation that is alveolar ventilation will decrease as the cycling rate is increased. Since in no instance can the mechanical impedance be entirely flow resistive it does not follow that the optimal frequency for a patient with high flow resistance is the lowest frequency possible. In such a patient the tidal volume will indeed increase as the cycling rate is reduced but a point will be reached when the elastic properties of the lungs and the chest wall limit further increases in tidal volume. The practical implications are these: Increasing the rate of a respirator will be detrimental rather than helpful if the principal difficulty is high flow resistance. Optimal rates in such circumstances will be in the neighborhood of 10 cycles per minute. At lower frequencies elastic properties will predominate in determining tidal volume even in instances where flow resistance is increased as much as tenfold above normal levels.

On the other hand increasing the amplitude of pressure will increase the effective ventilation whether the mechanical difficulty be primarily

elastic or flow resistive in character. In either case tidal volume will increase roughly in direct proportion to pressure amplitude until the range of volume change approaches the elastic limit imposed by the lungs and the chest wall.

In the supine individual the range of volume change from the relaxed mid position over which the compliance of the lungs and the thorax is approximately constant extends much further in the inspiratory direction than in the expiratory direction. This leads us to the question of the mean pressure level of the pressure cycle. It may readily be seen that as the amplitude of pressure is increased around a mean of atmospheric pressure the elastic limit in the expiratory direction is approached before that in the inspiratory direction. To take advantage of this the most compliant portion of the volume pressure curve mean pressure must be increased in the airway relative to the body surface. As a result of this the mid position will be increased and this will be favorable in terms of flow resistance as well since the flow resistance of the tracheobronchial system decreases as the average lung volume increases. However the most favorable mean pressure in terms of respiratory mechanics may prejudice venous return to the heart and the optimal mean pressure is a compromise between circulatory and respiratory effects.

In addition to the cycling frequency the amplitude, the mean level of pressure, we must include the implications of the shape of the pressure pattern and the timing of the phases of respiration. I should like to put my emphasis on the importance of the timing of the pressure patterns. It could be regarded as being, of more significance than merely the shape of the pattern per se.

Apart from questions of comfort two important factors argue for reducing the time of the inspiratory phase relative to the expiratory phase in the pressure cycle. When flow resistance is elevated because of bronchoconstriction or partial obstruction of the small passages within the lungs expiratory flow resistance tends to be greater than inspiratory flow resistance. This results from forces tending to collapse the air passages such as described by Dr. Whittenberger proximal to the regions of increased re-

sistance. Such collapse can be minimized by reducing the mean driving pressure during expiration and prolonging the expiratory relative to the inspiratory phase. A second advantage accrues from this to the extent that mean airway pressure is reduced thus minimizing the detrimental influence of pressure on cardiac return.

I have made no attempt to survey the methods of measurement available or the accomplishments in research in the field of respiratory mechanics. Simple methods have much to offer both in terms of results and in terms of your way of thinking and the results obtained can be of use in the management of the patient who requires mechanical assistance to ventilation.

Dr HEENSTRA. Dr Comroe has explained that an adequate ventilation of functioning alveoli is one of the main requirements for pulmonary gas exchange. Ventilation as such is a mechanical event and it is logical to expect that a knowledge of the mechanical behavior of the lungs and the thorax will help in understanding disturbances in the magnitude and the efficiency of ventilation and circulation in the lungs.

As Dr Mead has told us, relatively simple measurements can be very useful in determining whether the impedance to ventilation is primarily elastic or resistive in nature. Such measurements do allow certain conclusions regarding the optimal rate and pattern of pressure variations during artificial ventilation.

There is one point which has been already emphasized by Dr Whittenberger that may deserve further consideration: the question of the description of the elastic behavior of the thorax and the lungs in terms of absolute lung volume and of absolute pleural pressure. The choice of the level of respiration or in other words the expiratory lung volume during artificial ventilation is always to some extent a compromise between the most favorable conditions for the ventilation on the one hand and for the circulation on the other.

From the ventilatory point of view, a higher lung volume associated with a higher mean pressure level may be advantageous because both the total compliance of the thorax-lungs system is higher in this range and the flow resistance, especially during expiration, is lower.

From the circulatory point of view, high inflation pressures may cause a reduction in pulmonary blood flow both by their effect on venous return and by increasing pulmonary vascular resistance in part or all of the vascular bed. This effect, which is usually well compensated for in states of an adequate circulation, may become serious in states of poor vasomotor reactivity, low blood flow and circulatory inadequacy, whether they result from pulmonary involvement in poliomyelitis disease or from hypoxia or a rib cage derangement.

It has been reported that in such cases a reduction of the mean pressure level may be advantageous. Also a slight head low position may increase the circulation in poliomyelitis patients. However, the question is how far can we go in reducing the expiratory level of ventilation in severely paralyzed patients? In a supine position as Dr Whittenberger has remarked the weight of the abdominal contents pushes the diaphragm upward, especially when it is paralyzed. This may be associated with a reduction in the functional residual capacity. According to Mjorner and Svanberg, the functional residual capacity of artificially ventilated polio patients is reduced from 1.65 to 1.30 liters in the supine position. If a negative pressure phase is added, a further reduction to 1.04 liters occurs.

I would like to show here some of our own results with regard to the elastic behavior of the chest and the diaphragm and of the lungs in anesthetized normal and curarized rabbits (Fig. 765). The resting expiratory level was varied by shifting the tracheal pressure to the positive or to the negative side. The pleural pressure was measured directly and the volume changes of the animal were measured by a plethysmographic technique. Curarization combined with intermittent artificial inflation did lower the breathing level in this case but the phenomenon which I like to emphasize is the shape and the range of the pressure-volume curves. When the resting lung volume is reduced by the application of negative pressure to actively breathing rabbits, most pressure difference is taken up by the chest and the diaphragm, whereas in the paralyzed rabbits the pressure difference is taken up by the lungs. From a certain level onward the lungs of the curarized animals are apparently unable to diminish further in size, not because they are empty of air but because the conducting airways are collapsed. Here the lungs prevent a further decrease in volume of the passive chest. However, in the active state, increasingly more negative pressures are required to reduce the volume of the chest to low values. This is

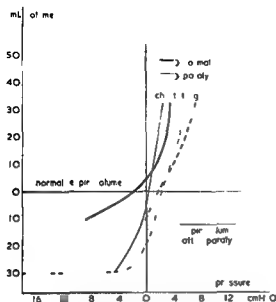


FIG 265 End-expiratory pressure volume relationships of the chest-cage (including diaphragm) and the lungs during spontaneous breathing and after muscular paralysis. Succinylcholine instead of tubocurarine has been used to avoid an eventual bronchoconstriction caused by a histamine liberating action of curare. The general conclusions remain the same.

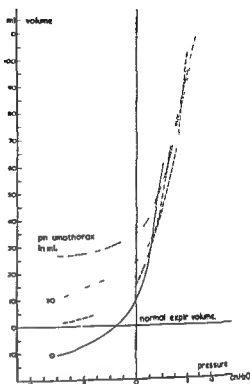


FIG 266 Chest wall pressure volume relationships in rabbits (means of 1 expts) at different lung volumes.

caused by the development of an active inspiratory tone which is still present in the state of expiration. Here the basic inspiratory tone of the chest and the diaphragm prevents the lungs from collapsing, the airways remain open even on application of considerable negative pressure.

We may conclude that the respiratory level especially when the lung volume tends to become smaller than normal is not simply the result of passive mechanical factors but that an active inspiratory tone of the chest musculature and diaphragm intervenes. This active tone determines the shape of the chest compliance curve in normal animals. It is a reflex tone brought about primarily by afferent impulses of collapse receptors in the lungs and mediated by afferent fibers in the vagus nerves. I shall show you in Figure 266 a number of chest compliance curves obtained at varying degrees of pneumothorax. Zero pneumothorax means pneumothorax of minimum size necessary to measure intrapleural pressure. Compliance curves of the

chest are shown with a pneumothorax of 15, 30 and 45 cu cm so you see the only thing which is different in this case is the volume of the lungs. The volume of the lungs determines the compliance curve of the chest.

In paralyzed patients the lungs may be deprived of such a reflex protection preventing a too great reduction of the expiratory lung volume and a collapse of the conducting airways. When the application of a negative pressure phase in patients with an already reduced respiratory level would indeed lead to such a collapse there is little doubt that regions of atelectasis and a pronounced unequal distribution of ventilation would develop.

A too great reduction of lung volume is perhaps also a danger in the application of mechanical coughing devices when the duration of the strongly negative phase is chosen too long. Theoretically I cannot see why such coughing devices should not be able to create a reduction in size of the intrathoracic air passages by creat-

ing a pressure gradient across the bronchial wall as in a natural cough. However it does seem possible that a collapse of the extrathoracic trachea could be produced also and that this would reduce the expiratory flow to such an extent that a narrowing of the intrathoracic airways would not occur.

Summarizing we have seen now that there is a level of optimal compliance and resistance where a given pressure change will cause a maximal tidal volume. There also exists a level where the conditions for the circulation are optimal and here one should be aware of the danger of a too low expiratory volume where the airways will collapse and regional atelectasis will follow.

In considering the optimal level of ventilation there is still another point which might be considered the efficiency of a ventilation of given size. This efficiency depends not only on the anatomic dead space but also on the equality of the distribution of fresh air and blood to different regions of the lungs. This again is to some extent a mechanical problem and I should like to discuss the question whether or not the efficiency of ventilation normally depends on active muscular factors which are absent in passively ventilated paralyzed patients.

The lungs are a structure attached at the hilus to a more or less fixed point in the thoracic cavity. The exact position of the lung hilus depends on the tension in the mediastinal connective tissue which extends to the pericardium and the diaphragm and on the other hand on the longitudinal tension in the trachea which is governed by the position of the larynx. From the hilus a framework of connective tissue radiates into the periphery of the lungs and attaches to the pleura. The mechanical tension in this connective framework creates the retractive force. This force is not necessarily the same at all regions of the pleural surface. It depends on the position of the lung hilus on the intrinsic characteristics of the lung tissue and on the shape of the thoracic cavity. There is now sufficient evidence for the existence of local differences in pleural pressure even in normal lungs. What we measure as esophageal pressure is probably near the pressure in the mediastinal or medial pleural space. This pressure at least in dogs is less negative than that in the

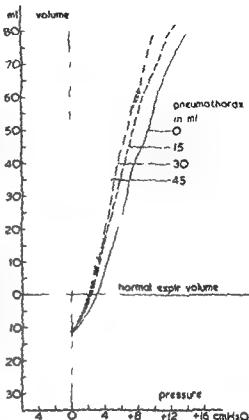


FIG. 767. Lung-compliance curves in rabbits (means of 6 expts.) measured at different pneumothorax volumes.

lateral pleural space. The matter of regional inequality of pleural pressure can be understood more easily if we realize that the free shape of the expanding lungs is not perfectly adapted to the shape of the chest in all phases of respiration. The degree of maladaptation determines the degree of pressure inequality. Maladaptation also increases the pressure necessary to achieve a given increase in volume but the effect on the distribution may be a more important one. If a spherical rubber balloon is inflated within a cubic box more pressure is needed than when it is inflated in a spherical box of the same content. If the balloon contained a spongelike inner structure also the distribution of the inflated air would be quite different in both cases.

Figure 767 shows the results of lung-compliance measurements in rabbits obtained during

pressure breathing with and without a pneumothorax. It can be seen that a compliance is larger in the presence of a large pneumothorax when the lungs are not forced into the shape of the adjoining chest wall.

Similarly changes in lung and thorax compliance have been observed by several authors after muscular paralysis. Apart from other causes such as the accumulation of secretions and regional atelectasis it would seem that this is at least in part due to differences in the inflation mechanism in the active and the passive state. Relatively slight differences in compliance when measured at an absolute level might be associated with relatively large changes in the distribution of ventilation.

The mechanical situation which I have tried to describe is compatible with the hypothesis of a co-ordination of respiratory muscular activity in normal subjects in such a way that a good efficiency of ventilation is achieved. In paralyzed subjects such a co-ordination may be deficient. This would explain to some degree the reduction of mechanical compliance and the decrease in efficiency of ventilation occurring in such individuals.

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DR COLLIER. If we are to treat a patient in trouble with abnormal CO and or O tensions should we increase the ventilation or give oxygen or should we do both? These are the prime questions that we have to answer.

Figure 268 is a diagram that we have found particularly useful in answering these questions. If we take point A as representing the alveolar oxygen and carbon dioxide tensions of a patient who is in trouble the alveolar carbon dioxide tension is 80 mm of mercury with a consequently reduced alveolar oxygen tension of approximately 50 mm of mercury. This patient we might choose to treat only by administration of oxygen. If we added 20 per cent oxygen to

the air which this patient was breathing this is a total of 37 per cent oxygen in the inspired air then the patient would move to point B. This would be good treatment for the hypoxia of point A but has done nothing to relieve the respiratory acidosis that the patient would still have and the CO tension will remain at 80 mm of mercury. A much more rational method of treatment for this patient would be to double approximately the alveolar ventilation and thus move the subject to point C which represents a carbon dioxide tension of approximately 40 mm of mercury and an alveolar oxygen tension of approximately 100 mm mercury thus tending to relieve both the respiratory acidosis and the lowered O tension.

However the problem as Dr Comroe has pointed out is that point C represents only the alveolar tensions for both carbon dioxide and oxygen. Problems of distribution or of diffusion lung pathology may cause the arterial-oxygen tension to be considerably below this value. However if we double alveolar ventilation again we would increase the oxygen tension only to 125 mm of mercury in the alveolar gas and this would be at the expense of producing respiratory alkalosis. A much more efficient procedure for a patient with abnormal lungs is to administer oxygen. We can see that a marked relief of the hypoxia can be expected and this then would be without disturbing the acid-base balance of the body. It is also interesting to note that on theoretical grounds Siskind and Rahn have pointed out that as CO tension goes below 40 mm of mercury the mixed venous O tension will actually decrease rather than increase so there may be little advantage in decreasing the CO tension below relatively normal values.

Ideally then we feel that the respirator should be set to maintain the arterial or alveolar CO<sub>2</sub> tension and the arterial pH at normal values. When this is done the arterial-oxygen tension and oxygen saturation will usually be satisfactory. However if adequate blood oxygenation is not achieved because of lung pathology then oxygen should be administered in sufficient amounts to overcome the defect that is present. Our studies on the arterial-oxygen tension in acute and chronic poliomyelitic patients indicate that while the alveolar arterial oxygen tension

difference is frequently greater than normal it is rarely necessary to add oxygen to the inspired air if the alveolar  $\text{CO}_2$  tensions is kept at normal values.

We have found that the alveolar analysis with the infrared  $\text{CO}_2$  analyzer has been of great value in the care of patients because we can rapidly evaluate the ventilatory status regardless of the manner of breathing whether with positive pressure curess the rocking bed the abdominal pressure cuff the glossopharyngeal breathing or breathing with the ordinary muscles. There is no apparatus dead space and the test requires no co-operation of the patient. The results are immediately available from breathes to breath and the arterial  $\text{CO}_2$  tensions can be estimated with accuracy in polymyocytic patients by this means. There is one word of caution depending too much on the arterial or alveolar carbon-dioxide tensions. It is only one of the three unknowns in the Henderson-Hasselbalch equation. In order to have a full understanding of the acid-base balance of a given patient one must know whether the pH or the serum  $\text{CO}_2$  content as well as the  $\text{H}_2\text{O}$  ever the presence of a metabolic acidosis or alkalosis frequently can be ruled out on clinical grounds alone so that we need do these additional studies only in questionable cases that are in trouble.

Some time ago we have used an automatic end tidal sampling method for obtaining alveolar gas for analysis. However, in respirator patients we frequently observed very poor results which we felt were due to the fact that some patients may have very deeply at tidal volumes due to swallowing or due to glottic closing during artificial respiration.

However, in patients with heart or lung disease the methods of direct alveolar gas analysis cannot be used. We have found the end tidal  $\text{CO}_2$  tensions in some cases to be as much as 30 mm mercury below the arterial tensions in patients with emphysema. This discrepancy of course is caused primarily by unequal distribution between the gas and the blood in the lungs in these patients.

It is fortunate that Dr. Harkness working at the Los Angeles General Hospital has worked out a rebreathing method using the method of Christensen, Douglas and Haldane which per-

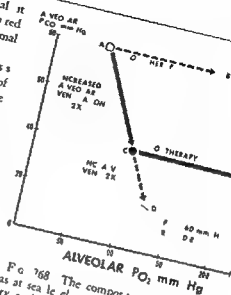


Fig 768 The composition of alveolar gas at sea level in a steady state. Respiratory exchange ratio = 0.8. Points A, C and D show the relationship between alveolar  $\text{PCO}_2$  and  $\text{PO}_2$  while breathing room air (A, C). Points B and E show the relationship between alveolar  $\text{PCO}_2$  and  $\text{PO}_2$  while breathing 80 per cent oxygen (37%  $\text{O}_2$ ) plus 70 per cent oxygen (37%  $\text{O}_2$ ).

mits an indirect estimation of the arterial  $\text{CO}_2$  tensions on the basis of this group of patients. A demonstration of the infrared analyzer in the rebreathing method can be seen in the exhibit section on the other building. It may be possible to adapt this technique so that a simple chemical analysis may be used and render the infrared analyzer unnecessary.

The arterial or alveolar  $\text{CO}_2$  tensions should not be used as a guide for placing the patient in the respirator. Whenever it is possible early enough to prevent rather than to treat hypoxemia.

In the absence of the methods of  $\text{CO}_2$  analysis we found that Radford's nomogram is quite satisfactory as a practical guide for setting the ventilation for the determination of the tidal volume. Ordinarily one can use any basal metabolic rate apparatus that one can find in an emergency hospital. However, a metabolic machine cannot be used readily when positive pressure is being applied to the airway and  $\text{CO}_2$  analysis is almost essential in this circumstance.



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We have found that the alveolar air analysis with the infrared  $\text{CO}_2$  analyzer has been of great value in the care of patients because we can rapidly evaluate the ventilatory status regardless of the manner of breathing whether with positive pressure cuirass the rocking bed the abdominal pressure belt glossopharyngeal breathing or breathing with the ordinary muscles. There is no apparatus dead space and the test requires no co-operation of the patient. The results are immediately available from breath to breath and the arterial  $\text{CO}_2$  tensions can be estimated with accuracy in polio-myelitic patients by this means. There is one word of caution in depending too much on the arterial or alveolar carbon-dioxide tension. It is only one of the three unknowns in the Henderson-Hasselbalch equation. In order to have a full understanding of the acid base balance of a given patient one must know either the pH or the serum  $\text{CO}_2$  content as well. However the presence of a metabolic acidosis or alkalosis frequently can be ruled out on clinical grounds alone so that we need do these additional studies only in questionable cases that are in trouble.

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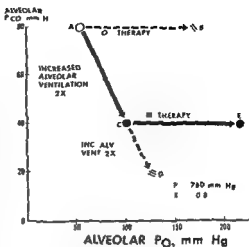


FIG 768 The composition of alveolar gas at sea level in a steady state. Respiratory exchange ratio = 0.8. Points A, C and D show the relationship between alveolar  $\text{PCO}_2$  and  $\text{PO}_2$  while breathing room air (21%  $\text{O}_2$ ). Points B and E show the relationship between alveolar  $\text{PCO}_2$  and  $\text{PO}_2$  while breathing 80 per cent air plus 20 per cent oxygen (37%  $\text{O}_2$ ).

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The arterial or alveolar  $\text{CO}_2$  tension should not be used as a guide for placing the patient in the respirator. Whenever it is possible artificial respiration should be instituted early enough to prevent rather than to treat hypoventilation.

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We feel that it is preferable to write a medical order for a given tidal volume and rate on a patient rather than to order a specific pressure setting. In this manner we can use a pressure-cycled respirator and retain many of the advantages of the volume-cycled respirator.

Almost all of our patients with acute poliomyelitis are treated either in the tank respirator or in the tank respirator with added tracheal positive pressure and it is extremely rare that adequate ventilation cannot be achieved with this equipment. In fact the majority of patients tend to be overventilated and we must exercise great vigilance if overventilation is to be prevented. We have studied a number of patients with chronic respiratory paralysis and long-standing hyperventilation. In a selected group of 14 such patients the mean arterial  $P_{CO_2}$  tension was 20 mm Hg and the mean arterial pH was 7.50 despite long-standing hyperventilation. The hyperventilation was corrected in all of these patients over a period of time by reducing the respirator pressures and in some cases by gradually transferring the patient to auxiliary respiratory aids such as the cuirass and the rocking bed. A most striking thing in this study besides the abnormal blood chemistry was the marked change in psychological pattern that we observed. By reducing the ventilation the patient was transformed from a complaining, whining, introspective individual into a patient with normal desires and interests outside of himself.

One patient with zero vital capacity has been markedly hyperventilated in the tank respirator for 4 years requiring supplemental oxygen much of the time. Repeated blood studies revealed an arterial  $P_{CO_2}$  tension of 12 to 14 mm Hg. However it was possible to reduce the ventilation gradually; the oxygen was discontinued and the patient was transferred gradually to a cuirass and a rocking bed. She was changed from a very severe nursing problem in the respirator center to a patient able to be cared for by her family at home.

We had thought that perhaps much of the acquired dependence on an increased ventilation such as in this patient was due to the new setting of the respiratory center. However certain other factors may be of importance. In a small series Dr. Crane of the Los Angeles General Hospital has observed that when the tank respi-

rator is opened and the hyperventilated patient is allowed to breathe spontaneously the alveolar  $CO_2$  tension rapidly becomes normal. When he is placed in the tank again the alveolar  $CO_2$  rapidly falls due to his subjective demand for the excessive ventilation. However when the cuirass or the rocking bed is used the patient's subjective ventilatory requirement is frequently markedly reduced from that required with the tank or positive pressure respiration. We do not understand the reasons for these differences.

In summary then we feel that extreme hyperventilation or hypoventilation are undesirable and we feel that we may prevent these only if we do have laboratory tests available to facilitate the institution of judicious treatment.

**PROF. MOLLARET:** I wish to speak of the circulatory disorders in poliomyelitis patients which it is necessary to be able to recognize although they are obscured by respiratory disorders. This subject has become a major preoccupation of mine. I will present some suggestive notes, a coherent physiopathologic interpretation and finally an experimental treatment of startling audacity for cases of poliomyelitis in imminent danger of death.

The first statement is that the study of circulatory disorders should henceforward take precedence over that of respiratory disorders. In fact the preceding communications suffice to show that theoretic knowledge in this field is already quite satisfactory; in addition a certain degree of mastery has been won in the treatment of such respiratory insufficiencies although this is true only of those who, following the example of Lassen, have not remained in the iron-lung stage. This is an apparatus which can save only a small number of poliomyelitis cases but can kill many.

The second statement is that the essential circulatory problem is found in the group of poliomyelitis patients dying of circulatory disorders despite the fact that correct respiratory equilibrium has been assured. If circulatory collapse (shock) was formerly frequent it was because the collapse was almost always secondary to a circulatory imbalance. Correction of the latter has reduced considerably this first category of circulatory difficulties, so true is this that in our center we have inverted the traditional findings looking to changes in pulse and arterial

blood pressure for danger signs of an incipient breakdown in respiratory equilibrium

The third statement is that the field of circulatory disorders not associated with respiratory difficulties is not unequivocal and that several factors should be distinguished. We will take only the last of these as the subject of our paper.

1 It may be a case of circulatory death from heart failure following cardiac arrest in this case there is nothing to be done but to open the thorax at once and to massage the heart directly. At our center everything is kept in readiness for this procedure and the number of patients we have been able to study under these circumstances already totals about a dozen.

2 It may be a case of circulatory death from primary collapse (shock) we are incessantly alert for the major premonitory signs namely a combination of slowing of the pulse and a rise in arterial pressure in the complete absence of hypercapnia. At this point cautious attempts can also be made to enlist the action of neuroplegic drugs. At the critical stage of the disease marked by tachycardia and an abrupt drop in blood pressure recourse to neuroplegics gives the coup de grace and the most one can do is to try and retrieve the situation by continuous intravenous administration of noradrenalin (the beneficial effect of which will but too often wear off despite increasing dosage). We shall not dwell longer on this form of collapse of non-respiratory origin merely contenting ourselves with adding that it may be associated either as a prelude or consequence with the third type of accident.

3 Or again in fact it may be a case of circulatory death from visceral hemorrhage above all from gastro-intestinal hemorrhage. Such a hemorrhagic process ultimately will be fatal in all cases neither perfect respiratory equilibrium nor even the most extensive transfusions nor recourse to constant supportive chemotherapy will save such patients. We wish here to describe understand and attempt to cure only this third type of circulatory complications in the light of investigations a preliminary account of which will be found in a recent paper written with various colleagues.\*

Consideration of these accidents is relatively recent and we have previously discussed the

case reports of Heyde and Robinson (1948 2 cases) Erskine Mason and MacDade (1950 2 cases) Cook Hartmann Sarnoff and Berenberg (1951 7 cases) Rundlett (cited by Cook 7 cases) Lenarsky Parr and Seanor (1951 1 case) Baker Cornwell and Brown (1952 7 cases) Horsey (1953 5 cases) Schaberg Hildes and Alcock (1954 23 cases) and finally those of Bennike and Grandjeant (1956). In the papers mentioned above we ourselves have presented a detailed discussion of 11 cases to which we can add 3 new cases (exclusive of cases other than poliomyelitis but distinctly showing evidence of the same mechanism and amenable to the same treatment).

We will not repeat the clinical description noting only that 3 types may be distinguished: (1) massive intractable hemorrhage fatal through acute anemia (2) moderate hemorrhage fatal through irreducible secondary collapse and (3) hemorrhage established only upon autopsy but having caused death before becoming externally manifest.

Instead we will briefly and very sketchily present our view of the histologic physiopathologic and therapeutic aspects involved.

But first one comment is indispensable namely that it is necessary for any interpretation to account for 2 other series of phenomena of the gastro-intestinal tract which may coexist in poliomyelitis. One of these is severe distention of the stomach and intestines which may have a very abrupt onset and likewise may be the forerunner of a fatal outcome the other is gastro-intestinal ulceration the recognition of which has been comparable to that of hemorrhage. Any adequate physiopathology must fit the explanations of the 3 phenomena into a coherent body of theory.

## HISTOLOGIC FINDINGS

Our histologic findings already seem to us to be very suggestive especially in regard to the unequivocal manner in which they indicate the existence of a common mechanism. We are able to reproduce here only some of the slides shown at the meeting which dealt with all of our cases nevertheless enough illustrations are given here to make apparent the following signs of hemorrhagic process studied



Fig 169 Extensive dilatation of venules  
slight arteriolar desquamation

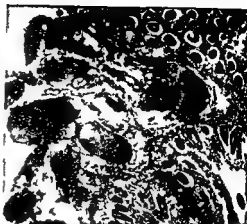


Fig 20 Enormous venous dilatation  
forming a spongy appearance



Fig 271 (Top left) Typical arteriolar  
venous occlusion almost complete arteriolar  
desquamation (Top left) Vascular  
Edema and dissociation

complete and non-inflammatory  
endothelial apical hemorrhage (Bottom  
vascular stroma (Bottom right))

- A First there are the direct vascular signs  
 1 In the capillaries congestion which might be considered unimportant  
 2 In the venules maximum vasodilatation far surpassing paralytic vasodilatation (Figs 269 2 0 271 top left and bottom left and 272) the venous walls may show no more than slight pathology their involvement being confined to a more or less desquamated endothelium Thrombosis is absent or rare and if it exists is minimally inflammatory (no polymorphs)

3 In the arterioles considerable desquamation of the endothelium ranging from simple detachment (Figs 269 and 271 bottom left) to obstruction of the lumen (Fig 271 top left) here too the lesion is minimally inflammatory

B There are in addition indirect or vascular signs  
 1 Opened hemorrhages are possible some times becoming great oozing patches (Fig 271 top right)

2 Edema of the submucosa may be extensive detaching large stretches of the layer and seriously impeding the nutrition of the tissues dependent on it (Fig 271 bottom left and right) we will only remark briefly that here we find the explanation of the genesis of those gastro-intestinal ulcerations whose importance is pointed out above

3 Finally there is swelling and hyperplasia of the lymphoid structures the hyperplasia also being noninflammatory Figure 272 shows a typical example

On the whole all our cases exhibited—in varying degrees but always unmistakably—such lesions all of which fall into the same category of completely noninflammatory but basically mechanical lesions This aspect was already known to us and we regard it as a specific type of severe vasomotor disturbance

# PHYSIOPATHOLOGIC INTERPRETATION

The authors we have cited as reporting clinical cases have not failed to postulate explanatory mechanisms we list these but only to reject them all  
 1 The role of respiratory dysregulation in particular that of anoxia (all of our patients exhibited normal respiratory equilibrium)

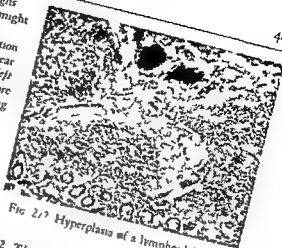


Fig 272 Hyperplasia of a lymphoid follicle

2 The role of a stomach tube (in our cases either it was not used or its use was discontinued)

3 The role of blood dyscrasia resulting from the poliomyelitis (this is the explanation of Bennike and Crandgeant we have never found any evidence of it)

4 The role of direct visceral invasion of the poliomyelitis virus (absence of any inflammatory process in the histology which is rather basically mechanical)

5 The role of vasomotor paralysis resulting from lesions of the vasomotor neurons of the brain stem the hemorrhagic syndrome thus becoming a deficiency syndrome as is paralysis of the extremities due to lesions of the neurons of the anterior medullary horns

To this list of explanations we oppose our own hypothesis which is that a vasomotor disturbance is indeed involved not however a deficiency vasomotor disorder but rather an irritative one It is not at all a question of a paralytic process but rather of an active sustenance process This will be of capital importance from the therapeutic standpoint since the indication will be not to excite but to paralyze or better still to block the nociceptive maintenance circuits

Here again we can only present our argument in outline referring to the paper cited and to forthcoming publications for further details  
 1 The histologic lesions are exactly those described by J Reilly as occurring in laboratory stimulation of the sympathetic nervous system  
 Mr Reilly's brilliant work since 1934 at the

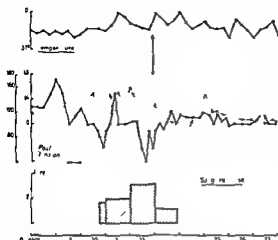


FIG 273 First case treated by bilateral prefrontal procainization. Immediate normalization of pulse and arterial tension (only the maximum pressure is shown)

Claude Bernard Hospital has elucidated this syndrome a bibliography of his works will be found in the paper mentioned before (see footnote on p 443)

2 *The histologic lesions and their progressive course* are duplicated in the events leading to the death of certain subjects of neurosurgery especially in operations involving the posterior cerebral fossa (Cushing De Marlet Clovis Vincent etc)

3 *The histologic lesions and their progressive course* are analogous to those so often described in the pathology of infectious diseases by Hutinel's designation malign syndrome the predominant lesions are analogous to those seen in our poliomyelitis cases (with due allowances for the diffusion of the lesions)

4 In addition *the points of agreement* between such an irritative mechanism and the vascular and paravascular lesions observed are easily understood

(a) *the desquamation of the arterioles* reflects the initial state in which there is extensive vasoconstrictions

(b) *the venous lesions* are a sign of active vasodilatation and evidence the enormous accumulation of blood in a veritable enormous visceral sponge this accumulation drains the cardiac pump and engenders fatal secondary shock

5 *The two combinations mentioned* are equally easy to explain on the one hand the severe gastro-intestinal dilatation (concomitant parasympathetic involvement) and on the other the ulcerations of the digestive tract (due to nutritional disorders brought about by lesions of the submucosa)

6 We may look to *therapy* for possible confirmation, a question which we will take up in our final section

### THERAPEUTIC TRIALS

Let us first recall very briefly the major finding stated at the outset of this paper the invariably fatal course of the hemorrhagic syndrome under investigation In particular all attempts at therapy aiming at stimulation of the vasomotor centers not only fail but hasten the process

Therefore when faced with another hopeless case we believed that we were warranted in attempting the inverse namely to aim at *paralyzing vasomotor activity* by direct pharmacodynamic blocking of the nociceptive maintenance circuits

*Direct bifrontal instillation of procaine after double trepanation* was our first attempt of this nature This was done for reasons detailed in length at the time particularly in order to act on the highest confluence of the neurovegetative system

The first case thus treated has been reported in detail in the paper cited before (footnote P 443) Although the patient died 47 days after the intervention (following a pulmonary infection due to *Pseudomonas aeruginosa* on the 26th day) the immediate benefit had been spectacular the charts in Figure 273 show the immediate and lasting normalization of pulse and arterial pressure The arrest of hemorrhage was also immediate (allowing for the time necessary for evacuation of blood accumulated in the digestive tract)

In a second case (still unpublished) death occurred as early as the 6th day but for other reasons and after the same immediate benefits had been noted The beneficial effects extended to a severe concomitant syndrome of dilatation of the digestive tract which receded almost instantaneously

Bilateral prefrontal procainization was not at

all our final measure we had also contemplated recourse to other methods

Our second approach was also to attempt high neurovegetative saturation but by the intrathecal route. This method was based on parallel examinations on the dog carried out at the Claude Bernard Hospital by J. Tardieu and J. J. Pouchot who acquainted us with the details of their experiments. We will only remark that 36 dogs whose nerve centres had been treated with procaine either by ascending spinal anasthesia (21 animals) or by intrathecal injection on following trepanation in the latter case either with (10 animals) or without (7 animals) pre-anaesthesia seemed to us to warrant a trial of such procedure in man. In fact the first patient in whom we tried this technique prompted by his hopeless state was a neurologist but not a polymyositis case (unpublished). This as a case of quadriplegia secondary to an injury of the upper cervical vertebrae in exploratory laminectomy resulted in no benefit and some days later a severe hemorrhagic syndrome set in associated with collapse and severe gastro-intestinal distention. An intrathecal injection of 0.5 per cent procaine had a remarkable effect on the abdominal distention but was unable to forestall death which occurred 18 hours later.

The third approach was much simpler: it was an attempt to see if the nociceptive circuit at the

lowest level of the nervous system i.e. the radicular by intraspinal procaine injection was made via the lumbar route and the patient was subjected to very carefully controlled progress. The first case thus treated (unpublished) was again not one of polymyositis but a woman with extremely severe postabortal tetanus. The patient had been tracheotomized and curarized the appearance on the fifth day of treatment of the severe neurovegetative disorder decidedly induced us to administer a slow intraspinal injection of 34 cc of 1 per cent procaine. The benefit was astonishing (unpublished case).

The next two cases were of severe polymyositis with the same neurovegetative syndrome. The same treatment was carried out and was also followed by immediate favorable results. We ourselves should like to stress that it is too early to speak of the ultimate survival of these patients however this does not detract from the fact that there was an immediate reversal in the condition of these patients both of whom had been despaired of.

We do not feel warranted at the present time in going beyond this statement. However we believe that no problem calls more urgently for thorough study than that of circulatory disturbances in polymyositis patients without respiratory sequelae. Perhaps the near future will bring confirmation of our working hypothesis from the physio-pathological as well as the therapeutic point of view.



# Swallowing Mechanism and Its Disturbances

DR JAMES F BOSMA

Disability of swallow is one of the most critical patterns of involvement of the acutely ill and of the persistently impaired poliomyelitis patient. The acute bulbar poliomyelitis patient with respiratory distress as a result of paralysis of the pharynx is a familiar clinical picture and one which deserves the most immediate therapeutic effort of the physician. The extent of motor disability of swallow is maximal at the time of the acute illness of poliomyelitis and immediately thereafter. Appropriately many subjects who have temporarily lost their swallow function during acute poliomyelitis recover it in the first days or weeks of convalescence. However there is a progressive accumulation of postpoliomyelitic subjects who have residual disabilities of this performance.

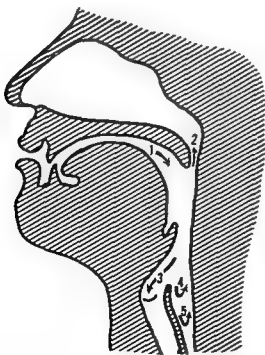


FIG 274 Poliomyelitic disability of the pharynx

A group of 34 patients child and adult having moderate or severe residual poliomyelitic disability of the pharynx have been observed by standardized clinical and roentgenographic procedures. The particular pattern of their disabilities has proved to be markedly varied. They may be pragmatically classified (Figure 2/4) as follows:

- 1 Impairment of control of penetration of the bolus from the mouth into the pharynx
- 2 Abnormal penetration of the bolus up through this palatopharyngeal isthmus and into the nose
- 3 Abnormal penetration in the airway
- 4 An arrest at the level of the hypopharynx
- 5 An arrest at the level of the entrance to the esophagus

In Figure 275 we see a bolus being taken by the tongue from the oral pool and being carried up to its preparatory position here. It is a function of the tongue to size the bolus correctly so that in successive swallows of material of light consistency the quantity collected by the tongue and sent down the pharynx is approximately similar.<sup>10</sup> The bolus is carried by the tongue to a swallow preparatory position where it is held by the tongue firmly against the posterior portion of the palate until it is ready to be sent down as the swallow. In order to hold the bolus there it is necessary that the tongue be of adequate volume that these muscles which suspend the tongue from the base of the cranium and from the mandible have a sufficient strength and also that the palate may be approximated toward the tongue.<sup>11</sup> All of these are required for an adequate control of the bolus in the swallow preparatory position and to prevent its premature entrance in the pharynx.

Figure 276 is a patient example in which two abnormalities of deglutition are shown. On the left note the bolus spilling without control from the mouth into the pharynx. In this patient



FIG 275 (Top left) Bolus being prepared for swallowing by the tongue



FIG 276 (Top right) Two abnormalities of deglutition



FIG 277 (Bottom left) Bolus enclosed normally within an indentation on the dorsal surface of the tongue

the reasons for this spill are (1) that the volume of the tongue is diminished (the dots on the illustration outline bolus which is penetrated into a trophic area of the tongue) and (2) the deficiency of suspensory mechanism of the tongue so the tongue cannot be held in position necessary to control this stream. A patient having this disability performs an unusual maneuver during eating, whereby he bends his head forward while he is masticating or preparing the material in the mouth and then he abruptly tips his head upward and backward to decant the bolus into the pharynx as an unquantified and unsized mass.

The bolus as it penetrates into the pharynx (Fig 277) is enclosed normally within an indentation on the dorsal aspect of the tongue.

At this time all other structures of the pharynx are raised and the palate is indented upward and backward. The patient shown in Figure 276 a) demonstrates the problem of nasal regurgitation. In Figure 276 right the bolus now is in the pharynx and part of it is penetrating upward between the palatopharyngeal folds and the postpharyngeal wall in the palatopharyngeal isthmus. The control of the palatopharyngeal isthmus depends on adequate motor strength of the palatopharyngeal muscles and also on adequate motor strength of the middle constrictor muscle of the pharynx which normally draws the lower border of the palatopharyngeal folds together. Asymmetrical weakness of the middle constrictor muscle of the pharynx may be identified by inspection of the pharynx through the open mouth during phonation (Fig 278). In this example the middle constrictor muscle on the patient's left side is weak. As we ask the patient to phonate we see that the posterior pharyngeal wall is drawn sharply toward his right (stronger) side. Thus is the curtain movement of Verret.<sup>2</sup>

In the circumstance of slight weakness of the palatopharyngeal muscles the palatopharyngeal folds may be sufficiently drawn together so that abnormal penetration of the bolus upward into

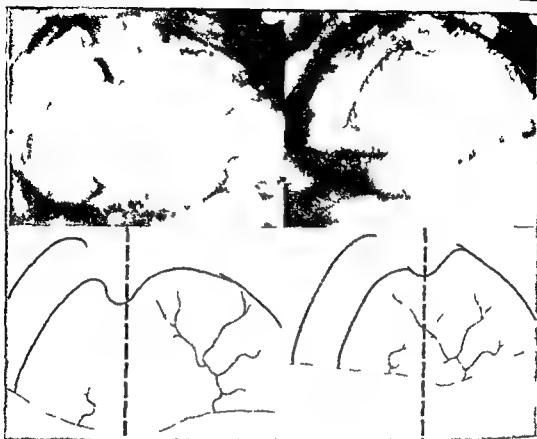


FIG. 28 Weak middle constrictor muscle on the left side of pharynx shown during phonation

the nasal pharynx does not occur. However, in the circumstance of weakness of the muscles of elevating the pharynx generally there may be a failure of approximation of the palatopharyngeal folds when other vessels approximate would occur. This weakness can be evaluated by placing the fingers of the examiner in the groove between the hyoid bone and the larynx (Fig. 29) to observe the strength of elevation of the pharynx during swallow. There is also a weakness of closure of the palatopharyngeal folds so that there will be a leak upward through the isthmus and possibly a nasal regurgitation of a part of the bolus.

Contracture of the sternohyoid and sternothyroid muscles is an abnormality of like effect to weakness of the elevators of the pharynx. We can evaluate this contracture by having the subject extend his head. In Figure 280 we see that in a normal subject extending his head

the hyoid bone and all structures related to are carried upward and remain in the same relative position to the upper cervical vertebrae. (In this roentgenogram the third cervical vertebra is outlined.) In the circumstance of contracture of these muscles the subject will suffer impairment of those functions of the pharynx which depend on adequate upward motion.

Now as the bolus penetrates through the pharynx and down into the esophagus the structures of the pharynx elevate still further (Fig. 281). The palate is angled sharply upward and the tongue and the hyoid bone are also at their maximal elevation. If there is weakness of the muscles suspending the structures of the anterior pharyngeal wall from the base of the cranium these structures may be displaced anteriorly and the anteroposterior diameter of the pharynx is created (Fig. 282). In these subjects the pri-

pal motion of the tongue the hyoid bone and the larynx during swallow may be forward or anteriorward instead of upward

The third possibility of abnormality of swallow is that of abnormal penetration of food into the larynx. The larynx is closed in swallow by the apposition of the arytenoid cartilages in the midline and elevation of the thyroid cartilage so that the arytenoid<sup>11</sup> and false vocal cords<sup>12</sup> are brought to proximity with the undersurface of the epiglottis. This arytenoid barrier to penetration of the bolus is not uniformly effective in normal subjects and by roentgen observation traces of bolus are at times seen to spill into the vestibule of the larynx though they do not penetrate through the false and true vocal cords to reach the trachea.

In subjects with poliomyelitic impairment of the complex musculature of the anterior wall of the hypopharynx there may be specific impairment of convergence of the arytenoid cartilages and the true and false vocal folds. Or there may be a deficiency of the more general elevation of the thyroid cartilage to accomplish approximation of the arytenoids and structures of the pharynx wall to the epiglottis. A penetration of bolus into the larynx may occur in the face of relative adequacy of the arytenoid valve if there is obstruction at the pharyngo-esophageal segment causing accumulation of bolus in the hypopharynx and its secondary overflow into the larynx.

In the pharyngo-esophageal segment there

are two distinct patterns of bolus obstruction. These are

1 Occlusion of the hypopharynx by a cricopharyngeal bar formed apparently by contraction of the cricopharyngeus portion of the inferior constrictor muscle

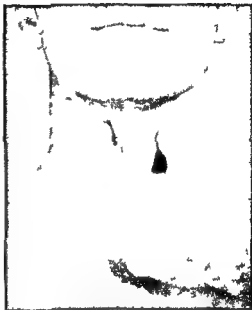


FIG. 279 Use of fingers in evaluating the strength of elevation of the pharynx during swallowing



FIG. 280 Elevation of pharynx structures when the head is extended in a normal subject.



FIG 281 Elevation of pharynx structures as bolus penetrates through the pharynx and down into the esophagus

## 2 Persistent closure of the sphincter at the orifice of the esophagus

The first of these patterns is found infrequently. In our 34 subjects who were studied

by reason of residual disabilities of pharynx following poliomyelitis we identified this roentgen demonstration is fairly difficult for the discrete bar of the cricopharyngeus portion of the inferior constrictor muscle is distinguishable but briefly during the swallow action protruding from the posterior pharyngeal wall after the first of the moving bolus has passed this level. In Figure 283 are example frames taken from cine fluorographic studies showing this discrete cricopharyngeal bar protruding sharply from the posterior wall to approximate the anterior wall.

The more common pattern of obstruction in this area is persistent closure of the circular sphincter at the orifice of the esophagus. The motor mechanism of opening of the hypopharyngeal sphincter in the normal subject is not completely understood. The sphincter is normally tightly closed and capable of resisting considerable increase in intrapharyngeal pressure. Its opening undoubtedly reflects inhibition of its circular musculature. It is noteworthy in this connection that in electromyographic studies of the musculature of the pharynx area in do



FIG 282 Increase in diameter of the pharynx due to anterior displacement of supporting muscles



FIG 783 Protrusion of discrete cricopharyngeal bar from posterior wall of pharynx

during swallow (Fig 784) a wave of inhibition was found to precede the sequential activation of the muscles in this sphincter area. In the human according to Negus<sup>18</sup> and Antoni<sup>9</sup> the co-ordination of the hypopharyngeal sphincter involves distinguishable somatic and sympathetic motor elements the latter from the superior cervical ganglion. The precise physiologic relation of the somatically and the sympathetically innervated musculature in the area of the sphincter of man requires further observation.

It is apparent that in usual modes of swallow elevation of the larynx is essential to this part of the swallow performance. With final elevation of the larynx as the bolus penetrates the hypopharynx the sphincter opens immediately and continually.<sup>9</sup> An active mechanism of opening of the sphincter has been inferred<sup>1, 10, 14, 15</sup> from anatomic studies of Terracol and Nijhet (Fig 285) in which a discrete midline bundle of muscle fibers was found to ascend from the anterior and the anterior lateral portion of the circular constrictor at the orifice of the esophagus to the posterior aspect of the cricoid cartilage and by careful dissections is found to send some fibers up toward the cartilages of Santorini. Negus has pointed out a relation between closing of the larynx and opening of the orifice of the esophagus attributing this opening to forward motion of the cartilages of Santorini by the underlying interarytenoid muscle.<sup>19</sup> This forward motion of the arytenoid cartilages has been observed by Ardran and Kemp<sup>5</sup> and also by ourselves in cinefluorographic studies of normal swallow. However in poliomyelitic sub-

jects having persistently closed hypopharyngeal sphincter the aditus may be well closed preventing penetration of the pooled bolus into the vestibule of the larynx. Apparently then this vertical and sphincteric motion within the arytenoid area is not uniformly associated with opening of the hypopharyngeal sphincter in man.

In the circumstance of persistent closure of this sphincter in 6 postpoliomyelitic impaired subjects the contour of the sphincter has been entirely normal and a catheter was found to penetrate in its midportion as in normals. The sphincter remained firmly closed during maneuvers of air inflation of the pharynx or combined inflation of the pharynx and the esophagus after air had been instilled into the esophagus by catheter. However others have reported success of such patients forcing food from hypopharynx into esophagus by air inflation of the pharynx.<sup>3</sup> Our study subjects were unable to swallow fluids or a catheter but could readily accomplish penetration of a catheter by the procedure of gagging. Appropriately they were able to vomit on occasion with apparently normal emesis stream. Recovery of penetration through this orifice was most typically abrupt and roentgen examination of satisfactory swallows later demonstrated normal contour of the open sphincter. Dilatation procedures are commonly ineffective in relieving this obstruction.

The possible explanations for this pattern of obstruction are several including

1 Deficiency of vertical traction from the

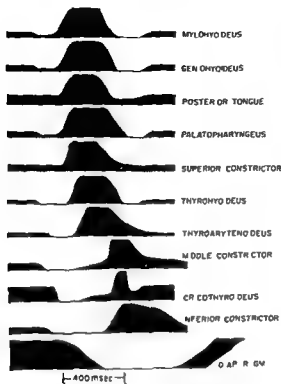


FIG 284 Musculature of the pharynx area in dog during swallowing



FIG 285 Midline bundle of muscle fiber (Terracol and Niche)

hyoid the thyroid and cricoid cartilages (See above)

2 *Contracture or fibrosis of the circular musculature of the orifice of the esophagus* This could well occur in association with a generalized syndrome of myopathy such as dermatomyositis<sup>1</sup> or myoporphyria<sup>16</sup>

3 *Swallow inhibitory influences from the mucosa of the hypopharynx or of the adjacent esophagus* The hypopharynx commonly demonstrates chronic inflammation in association with stasis of secretions.<sup>14</sup> Swallow inhibitory afferent effects may originate from chronically inflamed mucosae of pharynx or esophagus.<sup>7, 19</sup> It is of interest in this connection to note the exaggeration of gag reflex in these patients having complete incapacity of swallow. With resumption of adequate swallow the gag reflex subsides to normal in both its readiness of elicitation and its degree of response. There is no clear clinical evidence that the exaggeration of gag reflex and the absence of swallow are related phenomena though in neurophysiologic studies of patterns of motor co-ordination in the bulbar

area as elicited by stimulation of the supralaryngeal nerve it was found that the performances of swallow and of emesis were mutually exclusive.<sup>1</sup>

4 *A coordinative impairment of the act of swallow* resulting perhaps from impairment of the swallow regulatory nuclear mechanism. Sjoberg<sup>4</sup> has mentioned as a sequel of poliomyelitis the clinical situation of recent demonstrated atony of the esophagus with traction in the upper esophageal orifice also in the lower esophageal orifice. This attributes to bilateral poliomyelitic ablation of vagal nuclei an effect essentially identical with that which he had observed in experimental vagal transection in the cat in confirmatory experiments of Pavlov.<sup>9</sup>

5 *Functional disability in which dysphagia may be continued as an anatomically and physiologically inappropriate persistence of a disability*

We have gained increasing respect for functional aspects of disability of swallow are now engaged in a study of severely

phagic patients obtaining history of early feeding in infancy and observing their general attitude toward food and their physiologic response to food applied to mouth or directly to lower areas of the alimentary tract. We find the evaluation of the functional elements of the total swallow-disabilities situation to be most difficult and complex

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## DISCUSSION

DR ARDRAN Dr Bosma has summarized the work that he and his colleagues have done relating to the disturbances of the swallowing mechanism following poliomyelitis and has given an extensive review of the literature. With my colleague Dr F H Kemp and others we have investigated the problem on slightly different lines we have been concerned primarily with the behavior of the normal and its variations and have extended our work into the pathologic field only comparatively recently. Our experience of the problems following poliomyelitis has been broadly the same as Dr Bosma's; our criticisms of his work are primarily those of the interpretation of what is normal. We have had the advantage of investigating hundreds of normal and abnormal individuals by cineradiography at 25 to 50 frames per second as well as by other methods. Twenty-four patients with swallowing defects following poliomyelitis have been studied. It is important to obtain a full accurate history. We have seen several patients who have been described as having some difficulty in swallowing following poliomyelitis but by careful inquiry have found that these symptoms have been due to conditions present before the onset of poliomyelitis resulting from accidents, injuries or operations in the region of the neck. The inability to hold fluid contrast medium in the mouth without it leaking into the pharynx and some degree of pharyngeal residue may be due to the results of previous tonsillectomy.

Variations in the swallowing pattern may also be brought about by posture. All normal individuals in the erect position swallowing a fluid bolus approximate the anterior and the posterior pharyngeal walls and completely strip the contents into the esophagus. The epiglottis tilts backward as the larynx is elevated and the tongue of the epiglottis turns over the entrance of the larynx as the last of the bolus is stripped from the pharynx. However many individuals when swallowing with the head extended on the neck are unable completely to approximate the anterior and the posterior pharyngeal walls and a small pharyngeal residue may result. Although the epiglottis may be tilted backward

it is not completely turned down. The more viscous the bolus the greater the residue. One must distinguish between posture and paresis.

I would like to comment on Vernet's curving movement. The clinical sign that saying "Ah" on stimulation of the pharynx results in an upward and outward movement of the posterior pharyngeal wall has been assumed to be due to unilateral weakness of one of the constrictors. From our limited series we have reason to think that this phenomenon is due to asymmetrical action of the muscles of the soft palate since the patients who exhibit this sign do not always show definite evidence of unilateral weakness of the middle constrictor on swallow.

Dr Bosma seems to us to be unduly influenced by the work of Negus and Pressman who refer to the presence of 3 laryngeal sphincters, and by the assumption that elevation of the larynx and the approximation of the arytenoids to the base of the tongue play a part in its closure. We have shown that the laryngeal lumen from the level of the vocal cords upward normally closes from below upward and that this can occur without elevation of the larynx. This type of closure may be seen when singing, using glottal stop or coup de glotte and may occur when bearing down as in defecation or when the breath is held.

In Figure 286 bottom left the open larynx is shown black in this still lateral roentgenogram taken while the patient was quietly breathing. Figure 286 bottom right shows the same patient holding the breath with the larynx closed. The lower limits of the vocal cords can be seen clearly or can be in its original form. The lumen of the larynx is obliterated from the level upward to the entrance to the larynx. There has been no significant degree of elevation of the larynx. The upper sections left and right show the same thing taken from a cineradiographic film at 25 frames per second in which the laryngeal structures have been outlined with contrast medium.

In the normal subject during swallowing the larynx may be closed at any time during the phase of the descent of the bolus or even before



FIG 78C Laryngeal structures

the bolus has left the mouth but it is invariably closed as the bolus is being expressed from the pharynx. The laryngeal lumen is usually narrowed but still open as the first of the bolus passes the entrance to the larynx. Nonnutritive food or contrast medium may enter the vestibule and pass down to but not through the vocal folds and is usually expressed back into the pharynx as the laryngeal lumen is closed from below upward.

The 3 upper sections of Figure 24, show the bolus passing through a normal pharynx. In the upper left section the epiglottis can be seen outlined against the posterior pharyngeal wall barium spilling on either side of it into the hypopharynx. A small amount of barium can be seen in the vallecula and immediately below it a larger barium filled channel shows barium entering the narrowed lumen of the larynx as far as the vocal cords. In the original film this could be clearly



FIG. 287 Bolus shown passing through a normal pharynx

seen fanning out in the laryngeal ventricles. It is normal for the laryngeal vestibule to be open at this stage. In the upper center down by the left there has been further closure of the larynx and the contents of the laryngeal vestibule are being expressed upward into the pharynx. The upper right shows a later stage with the vestibule virtually empty. The patient was unaware that anything had entered the airway when this occurs; it is quite normal for the laryngeal contents to be expressed into the pharynx as the bolus is stripped from the pharynx. We would regard the presence of contrast medium in the vestibule as abnormal only when it was not stripped from the laryngeal lumen as the bolus left the pharynx. Figure 287 bottom right is a film from another individual showing how contrast medium has passed the entrance to the larynx while the lumen is still open. This is the

more usual state of affairs rather than that shown in the left hand side.

Those individuals who have trained themselves to "down a pint of beer" and who close the larynx while it is depressed do not show any abnormal laryngeal sphincteric movement; they merely have trained themselves to allow passage of fluid through the pharynx while the larynx as a whole is depressed.

The most important residual defect in swallowing following poliomyelitis is the inability to clear food from the pharynx. This may be due to several causes but in our experience the commonest cause is failure of the pharyngeal constrictor peristaltic wave; this may be associated with a failure to move the anterior structures (tongue, hyoid and larynx) sufficiently far backward. Although elevation and backward tilting of the larynx are not essential for its



FIG 288 Selected series of frames from a patient with pharyngeal palsy following poliomyelitis

closure they are important particularly in patients with a pharyngeal residue for two reasons. If the larynx is not adequately tilted backward there is a much greater tendency for food to enter the lumen. If the larynx is not adequately elevated this indicates a failure to shorten the pharynx in a vertical direction which leaves a much greater cavity for the retention of residue. That this is so has been seen in at least one patient who had attempted to commit suicide by cutting his throat. The wound had severed the pharynx between the hyoid and the larynx and had divided the constrictors at this level. Following surgical repair he was found to be quite capable of closing the lumen of his larynx and the pharyngeal constrictor wave passed downward with only a brief break but there was complete inability to elevate the larynx; this resulted in a large pharyngeal residue some of which entered the airway on re-opening the larynx. We have no experience of operations designed to assist eleva-

tion of the larynx in patients with pharyngeal palsy.

We have seen some individuals whose laryngeal sphincter appears to be capable of closing quite well but which closes too late after some contrast medium has entered the trachea. In our experience the commonest cause of material entering the airway has been spill from the pharyngeal residue as the airway is re-opening.

We also have observed what Dr Bosma has described as a failure of opening of the mouth of the esophagus. It is not quite clear just which muscle group is involved but we believe it is one of the bundles of the cricopharyngeus.

The cricopharyngeal bar referred to by Dr Bosma has been seen by us on many occasions and we are sure that Dr Bosma would have seen it much more frequently had he had the advantage of cineradiography at 25 or more frames per second. The contraction producing this sometimes appears before and sometimes after one would expect the peristaltic wave to have reached this region. We have seen patients

in whom dilatation of the cricopharyngeus has been of some help or even an operation in which the fibers are partly divided as in Ramstead's operation on the pylorus. We would regard cineradiography as an essential method to determine the precise nature and site of the defect before planning any operation.

Figure 288 shows a selected series of frames from a patient with pharyngeal palsy following poliomyelitis. In the first frame we see the lower pharynx following the swallowing of a mouthful of fluid contrast medium. There is a residue remaining. In the top left hand corner one can see the outline of the lower jaw and immediately to the right the rather dim outline of the cervical spine in front the hyoid bone and almost immediately below it the black shadow indicating the laryngeal airway. Barium residue can be seen coating the walls of the valleculae and lateral food channels and outlining the pyriform fossae and the pharynx immediately above the entrance of the esophagus. The next frame shows the larynx elevated and the next bolus descending. The laryngeal airway is still wide open. The third frame shows that the larynx is now closed. The next two frames show the bolus descending and being divided at the level of the oblique fibers of the inferior constrictor. The last frame shows the larynx refilling with air. A large residue remains in the pharynx the bulk of which will be swallowed by subsequent effort. No contrast medium enters the larynx. There has been no evidence of pharyngeal constrictor peristalsis; gravity and backward movement of the tongue and the larynx have been used. Later the forward movement of the tongue and the larynx have contributed to increasing the diameter of the pharynx allowing contrast medium to coat the walls. It will be seen that those patients who are unable to alter the size of the pharynx by maneuvers of the tongue and the larynx in the absence of constrictor peristalsis are placed in a most unfavorable position in dealing with a residue. It will be readily appreciated that residue might enter the airway on reinflation.

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# Glossopharyngeal Breathing Medical Management

DR CLARENCE W DAIL

Our first acquaintance with what we later called glossopharyngeal breathing was in 1948 a young man who had had poliomyelitis 4 years previously. We observed that each breath was associated with a strong pull of the neck accessory muscles which was immediately followed by one or more mouth and throat movements that appeared to be gulps of air. We found that the patient's vital capacity using his ordinary muscles of breathing was 150 ml but with the aid of these additional gulps of air he could raise his capacity to 600 ml. Soon after this experience we observed that several other patients had learned a similar method of breathing independently some by watching other patients. We recognized the basic principle of this method of breathing and since it consisted essentially of the use of the tongue and the laryngeal musculature we called it glossopharyngeal breathing. We saw a similarity between this method of breathing and that employed by amphibians and therefore also called it frog breathing.

The first of our patients to have learned glossopharyngeal breathing as an aid in respiratory muscle paralysis was a woman who had been paralyzed almost completely by poliomyelitis in 1933. She was first seen in our hospital in 1953 and it was observed that she used glossopharyngeal breathing in combination with neck accessory breathing. Her ordinary vital capacity was 200 ml but with glossopharyngeal breathing her capacity was 800 ml. At that time the patient resorted to that she had used this same type of breathing about a year after the onset of her disease that is in 1934 when attempting to free herself from the respirator.

The first normal person of whom we have any knowledge of having used glossopharyngeal breathing recalls that at about the age of 6 (in 1914) he used it as an aid in filling his lungs with air before diving. He is qualified to make this observation because in 1951 he contracted poliomyelitis and became a respirator patient.

When we began to teach glossopharyngeal breathing to this patient he told us of his previous knowledge of the method.

Since glossopharyngeal breathing has been used more extensively for specific aid in respiratory paralysis occasional reports have reached us of a patient here and there who has learned it without instruction and even without knowing that anyone else had ever used it. With our own patients we observed this in 8 cases. One could probably conclude that a certain small percentage of all patients who have marked difficulty in breathing will learn it on their own initiative. On the basis of this we might further assume that glossopharyngeal breathing probably has been inadvertently used for centuries.

Our first report of this breathing, was published in 1951. It appears the next report was published in 1954 by Kirschsieder who made an independent report of a patient who developed it following poliomyelitis. Kirschsieder compared the breathing of this patient with that of amphibians just as we had done.

## MECHANISM

We have studied the mechanism of glossopharyngeal breathing with the aid of cineradiography recording the pressure flow rate and volume changes as well as by visual observation of those who have learned it either normal subjects or patients.

The mechanism of glossopharyngeal breathing is essentially the same as that of a pump the tongue and the throat structures acting as the piston and the mouth the soft palate and the larynx acting as valves. There results a stroke or steplike expansion of the lungs as air is pumped into them. When inspiration is completed the inspired air is permitted to escape. The procedure has been described elsewhere in greater detail.

## PHYSIOLOGIC FINDINGS

We have made studies of the blood and the alveolar carbon dioxide tension the arterial oxy-

gen tension and arterial pH and have found that even after prolonged glossopharyngeal breathing these values are within normal limits. Thus the clinical observation that lung ventilation can be adequately maintained was confirmed by laboratory studies.

Glossopharyngeal breathing is an example of positive pressure breathing and consequently may be considered to impair cardiac function. For this reason intra arterial blood tension tracings were made during both quiet and deep glossopharyngeal breathing. Quiet breathing as used for maintaining ventilation did not produce an appreciable effect on blood pressure but some patients were able to produce a definite drop in arterial tension on expanding the lungs with a deep breath. This is borne out clinically. Deep glossopharyngeal breathing can be done without producing such effects and still greatly improve the effectiveness of coughing especially when manual assistance is also employed. Expiratory air flow rate studies done by us and by Feigelson confirm these observations.

### CLINICAL OBSERVATIONS

At present the number of our cases doing glossopharyngeal breathing has grown to 272. Of this number 8 learned the technic without any instructions or without any knowledge of the procedure. 37 might be considered as having taught themselves after a certain amount of observation such as seeing another patient doing it on the ward. The remainder 227 were specifically taught the method. 120 of this number were taught by 1 instructor. Of the 272 patients 54 could not breathe except by the glossopharyngeal method but with it could breathe an average of 4 hours and 57 minutes with a minimum of 1 hour.

Another group of 17 patients had an average breathing ability of 16 minutes. Most of the patients in this group were either taught early in our experience and had no follow up endurance training or were very recent cases who were still building endurance. Some had extremely weak glossopharyngeal musculature.

There was a group of 54 patients who made use of glossopharyngeal breathing as a supplement. Finally the largest number of patients (147) were taught the technic only for coughing and chest stretching.

### CASE PRESENTATION

The patient to be described illustrates some of the advantages, indications and management problems in glossopharyngeal breathing.

Four years ago at the age of 31 this mother of a 2 year-old child contracted poliomyelitis. She was admitted to our hospital 1 month later completely paralyzed except for the muscles of the eyes, the face, speech and deglutition. She had had a tracheotomy and received full-time artificial respiration with a tank respirator. The vital capacity was zero.

About 4 months after onset glossopharyngeal breathing instruction was started. 3 months later the patient had learned the technic but partly because of muscle weakness and partly because of a lack of a good follow up teaching program, it took another 9 months for her to build up her tolerance to 2 hours. At that time she had a glossopharyngeal capacity of 1150 ml. in comparison with a regular vital capacity of 225 ml.

About 1 month later she could breathe over 7 hours in one stretch but this was not easily maintained since mastication of food at meal times is not compatible with simultaneous glossopharyngeal breathing. There is also danger from choking. The usual breathing limit per session is 3 to 4 hours. Therefore this patient was put on a schedule of 3½ hours twice a day with other shorter periods for care as needed.

About 1½ years after onset of the disease the tracheotomy was repaired since she could now cough up her own mucus adequately. Incidentally no breathing time was permitted for several days after the repair.

The patient's vital capacity now is 260 ml and the glossopharyngeal capacity is now 1650 ml. The improvement in coughing is demonstrated by an expulsive force measurement of 0.9 cm water pressure without a deep glossopharyngeal breath and 41 cm with it. Her breathing is almost entirely by this acquired means although occasionally she does find it convenient to supplement with the neck accessory muscles.

About 1 year ago the patient was discharged to her home where she has a full time live in attendant. She uses a canvas respirator for sleeping and a rocking bed for rest and feeding.

She is taken for automobile rides as she

desires. She is pushed about in her wheel chair as occasion demands as for example for her weekly visits to the food market. She is a regular attendant at church and parent teacher association meetings. All this she had done thoughtlessly without any emergency resuscitation equipment along. When we found this out only 3 months ago we convinced her that our safety code requires that she always take respiratory equipment with her.

The patient lives a happy life with her child who is now 6 and going to school. She reads books considerably and is able to turn the pages with the aid of a properly designed book rack and a mouth stick. She does oil painting also with the aid of mouth and neck movements. If she desires she also can operate a typewriter by this means.

### TEACHING GLOSSOPHARYNGEAL BREATHING

In our experience glossopharyngeal breathing is best learned by a definite program of individual instruction. Our first patients had to learn by observation of others who had already mastered the technique. They also received occasional encouragement from the hospital personnel. Before we organized an effective teaching program 8 to 10 patients learned glossopharyngeal breathing each year. After we initiated the program the number rose to 55 then to 85. The teacher himself should know how to do glossopharyngeal breathing so that he can speak from experience and can demonstrate the steps in the technique.

A manual of instruction has been published. This is based on several years of experience in teaching and now about 3 years after preparation it still represents our basic method.

Glossopharyngeal breathing is obviously indicated as a substitute method when the patient has no expected functional return of normal breathing but still possesses strength in the muscles of the mouth, the throat and the larynx sufficient to perform the act. It is also indicated when the ordinary breathing muscles need assistance or rest.

Since glossopharyngeal movements can be employed to expand the lungs fully they are useful in improving a weak cough. Furthermore we believe that it is a valuable method of lung and chest stretching in all conditions that cause a

decrease in lung and chest flexibility or compliance. Although there is not positive proof of this clinical observations do indicate its value. When the patient expands his lungs sufficiently to cough or stretch there may develop enough intrapleural pressure to produce the Valsalva effect. It is our practice to instruct the patient how much to expand his lungs after checking the effect on the pulse and after noting any possible symptoms of fullness in the head, dizziness or other circulatory symptoms. Usually this is no problem and so far we have observed no evidence of injury.

It is important that the patient be enthusiastic about the program. Therefore he should be well oriented and fully informed about the advantages of learning. He should not entertain the thought that hope is given up for the return of normal breathing just because glossopharyngeal breathing is now being started. Actually it is an aid in the return of expected normal breathing.

A quiet environment should be furnished so that the patient can concentrate. There must be no situation which may cause emotional disturbance. The patient should possess confidence in learning but he should understand that it may take considerable time and effort.

If there is weakness of the muscles which are used the patient should be taught exercises which will strengthen them as well as build up endurance before instruction of actual glossopharyngeal breathing begins. The lessons should not be too long. 15 minutes seems to be the ordinary optimum time. Daily instruction is desirable.

The program for bodily activity or any other program which may tire the patient may have to be limited during the training period. This may include such procedures as freeing the patient from a tracheal respirator, use of a cuirass or (chest) respirator, gymnasium activity or surgery.

We have found that training a patient who has only a few minutes breathing tolerance requires the proper type of respiratory aid. One should be able to stop and start the aid instantaneously. For this reason we prefer either the cuirass, body type or mouth positive pressure respiratory aid.

Evaluation of a accomplishment during the course of training is necessary. Without a cer-



tain knowledge that the technic is effective much time can be wasted furthermore the student may have to learn tediously to correct a bad breathing habit. There are various ways of determining effectiveness. One should watch the chest and the abdomen to see if they are made to expand passively by small step movements. If the patient can cough and call definitely louder with the help of glossopharyngeal breathing this is further evidence of success. Improvement of vital capacity is also a good index. However it has been our experience that the best index of accomplishment is the spirometric tracing which shows steplike increase in lung volume. A suitable metabolism apparatus serves as a satisfactory spirometer. One must use a comfortable face mask which will not interfere much with the movements.

When a patient has learned the technic he may not yet have sufficient strength and tolerance to breathe more than 3 or 4 minutes by this method. There must be a continuation of diligence for it may take months before breathing can be sustained for more than an hour as was true in the case presented. All cases should be taught how to expand the chest deeply and also how to improve the cough by this method. Patients who are taught glossopharyngeal breathing for coughing should not forget to make it a practice to take several strong coughs daily so that the force is well maintained for the purpose of combating future respiratory infections.

If the patient has a tracheotomy tube he must be able to keep it stoppered for most of the time except when he needs suctioning. Excessive tracheal mucus may be a great hindrance to learning the technic of glossopharyngeal breathing. If it is still necessary for a tracheotomy tube to be used and the patient's tube is stoppered great care must be taken that the stopper does not pop out. The patient with respiratory paralysis thus may have no means of calling the attendant and no means of breathing.

### SUMMARY

The development mechanism certain clinical aspects principles of teaching and medical man-

agement of glossopharyngeal breathing are discussed. A case with respiratory paralysis is presented. When it is indicated and properly applied we have not observed any detrimental effect. In the absence of any appreciable weakness in the participating muscles patients have been able to learn the procedure in nearly every case although it may occasionally take many months. Its use has proved to be of gratifying value in many patients with respiratory muscle weakness or paralysis in furnishing independent breathing time as well as improving lung expansion and cough. In many patients it may have been shown to be of help in facilitating their care. There may have been several occasions when its use has appeared to be life saving.

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**DR. KIESCHIEPEL.** When a few years ago we studied replacement ventilation which was continuously observed in a polio patient (Fig. 289) we also had the idea as did Dr. Dail that there was a function which had been forgotten completely and could replace normal ventilation. Such an assumption might be somewhat romantic at first view because it includes a phylogenetic aspect. In this respect we should not forget that genetic history and viewpoints of this history have made a considerable contribution to our concepts on the development and the construction in the human organism. The spontaneous occurrence of glossopharyngeal ventilation as a replacement function and as a result of poliomyelitis paralysis of respiration is not the only proof that this auto-function remains within the realm of the human being. There are quite a number of observations which tend to prove that glossopharyngeal movements occur in the normal respiration. This is not only in agony or in the collapse of respiration where apart from shock respiration there might also be a deglutition particularly in premature children. Such movements also are found in narcosis and as a result of any hypoventilation. Further divers and swimmers know that such movements can counter the lack of respiration. Beyond that however healthy persons can learn glossopharyngeal breathing. Before we consider the problems involved let us look into amphibial breathing for comparison from a physiologic point of view.

However one cannot expect that the form of respiration is always in line with those of amphibians since in that type of animal there are also differences in the type of application of this respiration. Respiration through the skin plays an important part in that they have special epithelium for this.

Schematically glossopharyngeal breathing can be subdivided into 4 parts (Fig. 290).

1 When the nose is opened. There you have an oscillation which leads to renovation of the air in the mouth.

2 If you close the nose and open the valve you have the air pushed into the mouth.

3 If you raise the palate this air is pumped into the lungs that is inspiration. This is under B and C.

4 The valve is closed and the nose opened and there is a new ventilation in this way the respiratory mechanism starts all over again.

If the animal is excited only pressor lung respiration occurs. That is the upper graph as can be seen from the registration of the considerable oscillations (Fig. 291). If the animal calms down then the oscillations are seen in the lower graph. If the animal is completely quiet these oscillations are overwhelming and the synchronized occurrence of the considerable oscillations can be seen (Fig. 297).

A number of oscillations are registered which might be in line with the pump movements and with the respiratory work of our patient with glossopharyngeal breathing. The same applies to the supplementary breathing in the human being (Fig. 293) when (1) the air comes into the mouth by opening (2) the nose and the mouth are closed and during that period the larynx button is also closed (3) if the larynx is opened and the tongue and the muscles moved then you have them pumping in air into the respiratory circle then we have (4) the larynx closed again.

A number of pumping movements in the human being then lead to expiration. In the human being we have the same oscillations and pump frequencies which depend on the number of physical incentives and irritations. Now in a typical amphibian the pump-pressure respiration is dominant in the reptile the thoracic breathing is dominant. Of course this is not a clear-cut separation for instance the agouti solamentum shows signs where you can study the pump work. You never have thoracic breathing and glossopharyngeal breathing at the same time. It is only when you come to the higher class of animals that you have the separation clear-cut. This also leads to a stronger and stronger impediment to the lower layers which is only reactivated when you eliminate the central state. In this way the observations



FIG 289 Original respiratory curve (registered by the spiograph) of a 12 year-old child with glossopharyngeal breathing

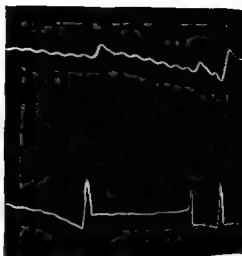


FIG 292 Original curve Synchronous recording of the thorax movement (top) and the guttural movement (bottom) in a frog

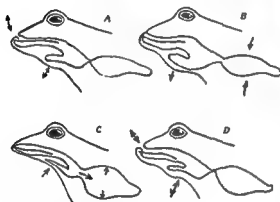


FIG 290 Schematic drawing of pump respiration of a frog (described in text)

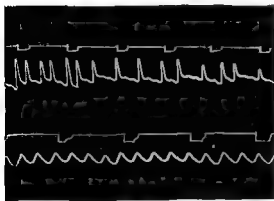


FIG 291 Original curve of the guttural movement and guttural oscillation of a frog (Top) Aroused animal Each guttural movement is bound with a pressurized lung ventilation (Bottom) Animal at rest The curve records additional guttural oscillations

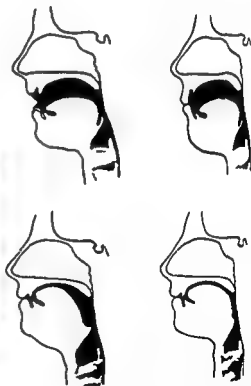


FIG 293 Schematic drawing of pump respiration in a human (described in text)

we have made on the deglutition and glossopharyngeal breathing can be explained. However we must not forget that the exact functional organization which leads to the glossopharyngeal varieties cannot be described in detail yet.

A few observations lead to the conclusion that it was not only the change of the  $\text{CO}_2$  tension in the blood that played a part but also general exercise can be possible and can lead to

glossopharyngeal breathing. The breathing mechanism generally occurs spontaneously only a certain period after respiratory paralysis but then can become automatic to such a strong degree that it makes not only eating and drinking possible but also is in line with sleeping. Even during sleeping after you stop the tank respirator you can stop it without having the patient notice it.



# Care of Patients Severely Stricken by Poliomyelitis

WEDNESDAY AFTERNOON, JULY 10, 1957

(This Session Convened in the Aula of the University of Geneva)

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PROF G FANCONI

University of Zurich  
Zurich

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Ann Arbor

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DR HORACE L HODES

The Mount Sinai Hospital  
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## Introduction

DR JAMES L. WILSON

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Only the enormous advances made in preventing poliomyelitis by active immunization can permit some contentment with the lack of progress in developing any form of specific therapy that influences directly or effectively the action of the virus in the central nervous system. About all we can do on a reasonably scientific basis during the acute stage of poliomyelitis is to make use of apparatus and technics not to influence the course of paralysis but to save life. In this Fourth Congress on Poliomyelitis as in the preceding ones the problems of saving life by mechanical technics have dominated the session on the care of patients in the acute stage of disease. The same emphasis will be made in this program but we hope that considerable attention will be given also toward making this saved life a more useful one.

To a great extent the problem of saving life with only paralysis of respiratory muscles remaining has been successfully met. To a lesser extent but still with considerable success the problem of saving life has been solved in patients who with or without respiratory muscle paralysis have difficulty in breathing because of the paralysis of their pharyngeal muscles. Limited progress only has been made in handling patients with acute brain stem disease that may disturb not only the control and efficiency of respiration but also the vasomotor system with resulting abnormalities in blood pressure, pulmonary edema and other manifestations of shock. Studies of these victims at autopsy with appreciation of the vast extent of the damage done by the virus makes us realize that it is improbable that any therapeutic technics can be successful in these patients until an antiviral agent has been found.

In any consideration of these lifesaving procedures certain moral problems continuously arise as they did in fact with the first patients with respiratory muscle paralysis who were treated with prolonged artificial respiration. The question does arise whether a physician has a

duty or even a right to save the life of a patient doomed to permanent and complete helplessness. Most physicians do not believe that they should accept the responsibility for euthanasia even by inaction. But in any case the clinical course of poliomyelitis shows such great spontaneous variations that a dependable prognosis in the acute stage is impossible. Patients who at first seem most disastrously paralyzed do sometimes show remarkable recovery so that it is impossible for a physician who might wish to make a decision in the acute stage of poliomyelitis as to whether his patient's life was worth saving or not, to do so with any certainty.

In the past many of us have given altogether too little attention to the after-care of the life which we have saved by artificial respiration during the acute stage of poliomyelitis. This has been particularly true in the United States, where many lives have been saved in the acute stage by the efficient use of the widely available tank respirator but such patients have been left dependent upon this device for months or many years or even for the rest of their lives. Much progress has been made recently toward success in returning these patients to their homes free of cumbersome mechanical aids to respiration. Few patients with respiratory muscle paralysis, saved from death by artificial respiration need to continue permanently dependent upon such apparatus for respiratory assistance. Such a patient almost certainly can be freed from hand-capping apparatus for most of the hours of the day at least and usually can be returned with care to his home by persons without technical skill or training.

But saving a life and thus freeing a patient to a great extent from dependence on artificial aids to respiration does not in fact complete our medical obligation to this tragically crippled person. We may all believe that each is born with the right to life and personal liberty but there is another right which perhaps should be

accepted as equal to these one to which the medical profession can give great assistance that is the right to be useful. This ultimate in rehabilitation must not be neglected by physicians. How can we make our patients who have been freed from respiratory aids also become useful perhaps to earn a living certainly to take care of their own persons and possibly their homes and to get further training and education? We feel happy that great progress has been made in attaining such ends with extensively paralyzed patients and although at

times this has been accomplished only at great cost, it is a cost quickly repaid by a few successes. When two thirds of a group of quadriplegic patients who have been confined to respirators for many months have been able with skilled assistance to progress to a point of earning an honest living we can claim important progress even though it may be pathetically little as compared with the prevention of the disease or the hope that sometime we actually may cure it during the acute stage when prevention has not been accomplished.



## *Statistical Considerations of Mortality and Prognosis of Patients with Respiratory Disturbances*

DR H C A LASSEN

In the acute state of poliomyelitis and during the first few weeks after the most frequent cause of death is respiratory insufficiency with its complications: obstruction of the airway, atelectasis and pneumonia, pulmonary edema and circulatory shock with kidney damage and quite often also signs of impairment of deglutition and symptoms of involvement of the autonomic centers in the brain stem. In some few cases death can be ascribed to polioencephalitis with hyperpyrexia.

It seems surprising that so relatively little has been published regarding the particulars of the complex clinical pictures of this disease which endangers life and practically nowhere has any attempt been made to correlate death rates with treatment. This no doubt is due to the fact that until recently therapeutic results in the severe forms were uniformly disappointing if not downright discouraging.

However this gloomy picture seems to have changed somewhat for the better in recent years. But still we are far from sure that our improved understanding of the underlying pathophysiologic conditions and our more intelligent interpretation of biochemical effects—which have given rise to better therapeutic equipment—can be made responsible for this amelioration of prognosis. In fact this is to be doubted.

An immense number of data on death rates and on the incidence of the different clinical forms of poliomyelitis are on record. In many countries notification has been obligatory for decades. Most often death rates are computed on the basis of the total number of cases notified which of course is misleading. Fatality rates should be based only on paralytic cases because first death occurs only in patients with paralysis and second while the diagnosis of paralytic poliomyelitis can be assumed to be fairly correct—especially in epidemic times—the diagnosis of nonparalytic meningitic poly-

myelitis not to mention the milder forms is often impossible to make with any certainty. It follows that fatality rates based on such data can never be even reasonably correct. Only the health authorities in Sweden, the United Kingdom and Denmark distinguish between paralytic and nonparalytic cases.

Besides the importance of correct diagnosis and conscientious notification other factors influencing statistics should be kept in mind before a fair comparison of data from different countries and at different times is attempted.

Fatality rates are usually higher in endemic than in epidemic cases. Males generally have higher death rates than females especially in the older age groups and as a whole mortality increases with increasing age in both sexes. Finally poliomyelitis like infectious diseases in general is never static. The epidemiologic pattern is steadily changing from the primitive type in which clinical disease is quite rare and mostly appears in small children toward the type now prevailing in countries with a long experience of poliomyelitis in which the disease seems constantly to move upward in the age groups.

From clinical experience we know that the severity of epidemics varies considerably. Even virus strains belonging to the same type may show great differences of virulence in laboratory experiments and this no doubt must play a role in the epidemicity and the severity of the disease. However this feature is difficult to assess and can be described only objectively by stating the incidence of life threatening complications and their relation to mortality.

In Table 157 the relation between mortality and certain complications is evident. Some of these complications especially hyperpyrexia and probably edema carry high mortality rates. These figures are from the epidemic in Copenhagen in 1957 where we had 1,235 paralytic cases including 333 with respiratory insufficiency with or without impairment of deglutition.

TABLE 157 COMPLICATIONS INCIDENCE AND RELATION TO MORTALITY  
(345 CASES—MORTALITY 47 PER CENT)

COMPLICATIONS	INCIDENCE		MORTALITY	
	NO. OF CASES	PER CENT	NO. OF CASES	PER CENT
Shock	134	39	90	67
Hyperpyrexia	66	19	60	91
Uremia	36	22	28	78
Pulmonary edema	29	8	26	93
Paralytic ileus	112	32	43	44
Hypertension	65	19	27	42

Only patients age 15 and over

These features all combine when characterizing epidemics and are of decisive importance to mortality. In other words spontaneous differences are so great that critical evaluation of one or another type of treatment in respect to mortality is extremely difficult if not impossible at least if the life-threatening ones are not a carefully described and carefully grouped.

We need an intelligent classification and we ought to try to find one on which we all can agree. Such a classification should be simple, unequivocal and sufficiently detailed on all decisive points. We should state our diagnostic criteria and our therapeutic indications clearly. The usual anatomic classification obviously is not satisfactory.

As an unobstructed airway prognostically is of the greatest importance in cases with reduced alveolar ventilation the simplest way of grouping these patients is to divide them into two categories: dry and wet. Wet is here defined as accumulation of secretions in the lower respiratory passages. Therapeutic indications are different in these two categories and the prognosis

is aggravated significantly in the wet group.

Apart from diagnostic criteria which we ought to find a way of standardizing an intelligent description of therapeutic indications is equally important before we attempt to compare the effect of different therapeutic measures such as indications for doing a tracheotomy for the use of tank respirators or for the use of an intratracheal positive pressure machine. If tank respirators are used on wide and not specifically stated indications on patients solely designated as respirator patients results naturally will be comparatively favorable.

Yet even when most of the points hitherto mentioned are taken into account comparison of series of patients from different clinics is still extremely difficult probably because we cannot describe adequately the severity of the disease. This is well illustrated by comparing fatality rates in the Copenhagen epidemic in 1957 with the figures published by our Swedish colleagues in their brilliant study of the Stockholm epidemic in 1953. Their diagnostic criteria and therapeutic indications were very much

TABLE 158 DEATH RATES OF PATIENTS CORRELATED WITH TYPES OF CASES

COPENHAGEN 1955			STOCKHOLM 1953	
	NUMBER	FATALITY RATE PER CENT	NUMBER	FATALITY RATE PER CENT
Deaths	102		27	
Paralytic cases	1130	9.0	633	4.3
Respiratory insufficiency	205	48.3	144	18.8
Respirator treatment	154	54.5	89	30.3

TABLE 159 EVOLUTION TIME OF RESPIRATORY INSUFFICIENCY IN 333 PATIENTS

EVOLUTION TIME	NO OF PATIENTS	DEATHS	MORTALITY RATE PER CENT
Less than 2 days	160	85	53
More than 2 days	149	50	34
Not established	24	6	(25)
Total	333	141	47

like ours and the epidemiologic pattern of the disease is nearly identical in the two countries.

In Table 158 death rates are correlated with the number of paralytic cases, the number of patients with respiratory insufficiency and the number of patients who had respirator treatment. Fatality rates in all groups were highest in the Danish epidemic although we had practically the same indications in the two countries. This to some extent must be due to a greater severity in the Copenhagen epidemic even if such a difference is not proven. Yet it is possible to assess the effect of the therapy introduced by us in 1952 by comparing the fatality rates in our hospital before and after the new therapeutic measures were introduced. Before this the fatality rate was over 80 per cent at the end of the epidemic; it had fallen to 25 per cent despite seemingly unabating severity of the respiratory cases.

The time of evolution of respiratory insufficiency has some bearing on prognosis. Table 159 shows that when respiratory failure develops rapidly the prognosis is less favorable than in cases with a more protracted evolution time. The duration of artificial ventilation—here called ventilation time—also is related to prognosis.

From Figure 294 it will be observed that in about 30 per cent (56 out of 194) of the cases artificial ventilation was not necessary. As to the remaining 138 patients, the curve indicates that within the first 2 or 3 months of artificial ventilation the individual patient still has a good chance of regaining adequate spontaneous respiration. After 6 to 9 months the prospects of regaining full spontaneous respiration are only slight. Thus of the 102 patients who after 1 month were still in respirators, no less than 43 had to be given continuous artificial ventilation, including 25 who eventually became chronic.

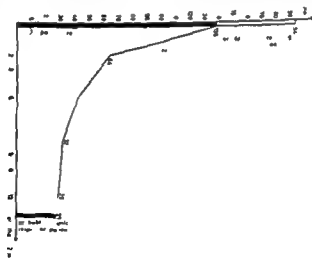


FIG. 294 Chart of patients with respiratory failure showing number of those requiring artificial respiration, number not requiring artificial respiration, and number of chronic respirator patients.

respirator patients. These patients all had severe paralysis of the diaphragm and the intercostal muscles. However it is worth stressing that the diaphragm may start regaining contractility very late. In some cases it may take 5 to 6 months before active contractions commence but even then normal function can be attained.

Our 25 chronic respirator patients have been ventilated continuously by means of intra-tracheal positive pressure machines and 18 are still in the hospital nearly 5 years after the acute stage. Three have died: 2 in hospital, 1 quite suddenly from cardiac failure and 1 from calculus pyelonephritis but none from pulmonary complications. The majority have renal calculi and intractable infection while stone formation is quite rare even in severely paralyzed patients who have not had artificial ventilation. A few have been operated on but in most cases the stones constantly grow although in some few instances they have been observed to disappear spontaneously. Probably most of these unfortunate eventually will die from uremia.

Since vaccination was started in Denmark in 1955 on a wide scale—98 per cent of the children and more than half of all adults have now

had at least two injections—we have had little poliomyelitis and only 1 respirator case in an unvaccinated girl. Whether this is due to the vaccination I do not know. Yet from other evidence I feel confident that—now at long last—thanks first to the brilliant work of our American colleagues we can see the end of respiratory insufficiency in poliomyelitis.

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## *Statistical Considerations of Mortality and Prognosis of Patients with Respiratory Disturbances*

DR HORACE L. HODES

I will discuss in part along the lines which Dr Lassen started. A discussion of statistics regarding the mortality and prognosis of patients with respiratory disturbances must take into account the fact that poliomyelitis may bring about respiratory difficulty in a number of different ways. These difficulties we list generally as four:

- 1 That caused by injury of the nerve cells in the medulla which control respiration.

- 2 That due to obstruction of the respiratory tract which is caused by weakness of the muscles of swallowing.

- 3 That due to impairment of functions of the respiratory muscles themselves.

- 4 Finally common and most important various combinations of all of these first three types.

Prognosis is different in each of these categories both for survival and for ultimate freedom from mechanical respiratory devices. For a number of reasons prognosis for immediate survival is poorest among patients with a central respiratory disorder. For example the central control of systemic and pulmonary blood pressure is often severely disturbed in these patients so that death from shock or from pulmonary edema may occur even when adequate pulmonary ventilation can be furnished to the patient by mechanical means.

With modern methods of treatment survival is the almost universal rule when respiratory difficulty is caused by obstruction of the airway due to weakness of the muscles of swallowing that is provided that this is the only cause of respiratory difficulty. As for the muscles of respiration themselves a rough index of the prognosis for patients with this disability can be obtained by serial measurements of the vital capacity. For example a survival rate of about 100 per cent may be expected among patients whose vital capacity in the acute phase of uncomplicated respiratory muscle poliomyelitis

does not fall below 75 per cent of the expected normal value. Furthermore such patients rarely need mechanical respiratory apparatus. Patients whose vital capacity does not fall below 50 per cent are in a similar position provided that their breathing difficulty is due entirely to respiratory muscle weakness.

When the vital capacity does fall below 35 per cent of normal the patient may be in danger of dying unless artificial respiration is provided. In attempting to give a prognosis in a specific case we must realize that the vital capacity is only a rough guide to prognosis and that many other factors are involved. For example a patient may be able to meet his respiratory needs with a vital capacity of 30 per cent if this ventilation is achieved entirely by motion of the diaphragm. In contrast a patient with the same vital capacity which is achieved by contraction of accessory muscles of the neck may be able to breathe unassisted for only a few hours. Prognosis then is different in each of the two cases.

During the past 10 years improved medical care has reduced the case fatality rate greatly among poliomyelitis patients with respiratory difficulties. The chance of attaining freedom from mechanical devices also has been improved greatly as Dr Wilson said. These changes are due to a number of factors and improvements.

The prognosis for poliomyelitis patients with respiratory impairment has improved in the last 10 years. The question is can we give a more accurate estimate of the prognosis than this encouraging generalization? An accurate estimate is beset with difficulties some of which I shall consider briefly.

First the outlook for a given series of patients will depend to a large degree on the number of patients with central respiratory disease in the group. The result attained with a series which includes a high percentage of such cases

### 2 YEAR EXPERIENCE WITH 500 ACUTE POLIO RESPIRATOR PATIENTS

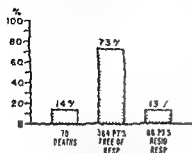


FIG 295 Two year observation of 500 consecutive respirator patients indicating mortality respirator freedom and residual respirator incidence (Affeldt J E *et al* Paper presented at meeting of Respirator Center Committee of National Foundation for Infantile Paralysis New Orleans 1956)

will be much worse than that with a group made up almost entirely of patients with respiratory muscle weakness. A second important variable concerns the difference in the criteria used for deciding whether or not respiratory involvement is present. For example one investigator may search carefully for minor degrees of respiratory muscle impairment and he then may include patients with minimal respiratory loss in his statistical study. A second physician may fail to detect slight respiratory muscle impairment or he may exclude such patients from consideration deliberately on the grounds that they are not in danger. The overall prognosis will appear to be much better for the patients of the first investigator than for patients in the second group. Care must be taken to avoid attributing such apparent difference to a particular form of treatment or indeed to a particular special type of apparatus. Finally the skill and experience of the physicians, nurses and other attendants have a direct bearing upon prognosis and a team meeting its first epidemic of poliomyelitis will not do as well as will one with previous experience.

Now the variable factors which we have discussed would tend to be cancelled out in a large series of patients. A study of such a group carried out with criteria as uniform as possible should yield an accurate estimate of over-all prognosis. Such studies have indeed been carried

### TIME PATTERN OF 364 PATIENTS WHO BECAME FREE OF ALL RESPIRATORY APPARATUS

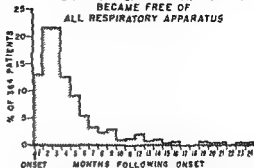


FIG 296 Three hundred sixty four of the 500 respirator patients became free of all respiratory equipment within 2 years of onset. The time pattern for this is presented by 6 months 83 per cent of the 364 had become free (Affeldt J E *et al* Paper presented at meeting of Respirator Center Committee of National Foundation for Infantile Paralysis New Orleans 1956)

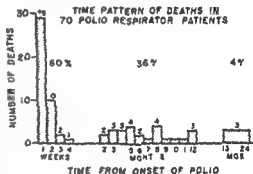


FIG 297 Seventy out of the 500 patients (14%) died within 2 years of disease onset. Over 50 per cent of the fatalities occurred during the first 2 weeks (Affeldt J E *et al* Paper presented at meeting of Respirator Center Committee of National Foundation for Infantile Paralysis New Orleans 1956)

out in a number of places. One was carried out by Dr Affeldt and his colleagues in Los Angeles and one by Spencer and Jensen in Houston.

Affeldt's study is concerned with patients with severe respiratory difficulty and it can be assumed that the results which he reports are less favorable than those which we might expect

with less severely affected patients. Affeldt studied 500 consecutive polio patients who were placed in tank respirators in the Los Angeles County Hospital the study running between January 1 1950 and August 15 1953.

At the Los Angeles County Hospital the vital capacity is routinely measured for acute polio patients. A poliomyelitis patient is placed in a tank respirator if his vital capacity falls to approximately 30 per cent of the predicted normal. This means that nearly all of the 500 patients in Affeldt's study had a vital capacity of 30 per cent or less of the predicted normal on admission to the hospital or soon after.

Affeldt's patients were observed for a minimum of 2 years and his over all results are shown in Figure 295. Of the 500 patients listed 70 died a case fatality rate of 14 per cent.

Sixty six or 13 per cent of the patients were still using respiratory equipment 2 years after the onset of poliomyelitis. Some of these 66 patients required the respirator some a rocking bed and others only a cuirass respirator. Of the 66 patients still using mechanical devices 27 employed these only at night. These 66 patients

included those who used the respirator part or all of the day. We see that 364 of Affeldt's patients 73 per cent became completely free of all mechanical respiratory equipment. Freedom from respiratory devices was attained most often during the second and third month after onset of illness. Eighty three per cent of those who eventually became free of mechanical devices had done so by the time 6 months had elapsed. However 1 or 2 patients each month achieved freedom up to 2 years after onset. This is shown in Figure 296.

Well we see that a vast majority of patients became free of respiratory difficulties by about the sixth month. There is a scattered improvement up until the period of 2 years. After that time freedom from respiratory equipment of all kinds is much less likely to occur.

Among Affeldt's patients death occurred most frequently during the first week of disease. About half of the 70 fatalities were experienced during this period. Of all the deaths which took place during the 2 year study 60 per cent occurred during the first 4 weeks of illness. Thirty six per cent of the deaths occurred from the second to the twelfth month and 4 per cent

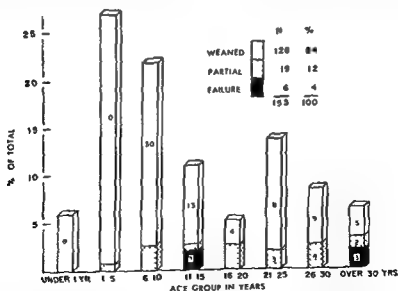


FIG. 298 Respiratory outcome of 153 acute poliomyelitis respirator patients requiring more than 2 weeks of respiratory assistance (Spencer W. A. and Jackson R. R. Paper presented at meeting of Respirator Center Committee of National Foundation for Infantile Paralysis New Orleans 1956)

occurred between the thirteenth and twenty fourth month as shown in Figure 297

Affeldt's results are similar to those obtained by Spencer and Jackson although the data were arranged in a somewhat different manner in the two studies

Spencer and Jackson's data (Fig. 298) deal only with patients who required tank respirator treatment for a period of 2 weeks or longer. Furthermore patients who needed respiratory aid for less than 2 weeks were excluded and also those who died within 2 weeks after contracting poliomyelitis. Altogether these authors studied 153 respirator patients who were treated by them from the onset of the illness. The patients in the group were observed from 6 to 76 months with the great majority being studied over 1 year.

Of the 153 patients 178 (84 per cent) became entirely independent of all respiratory devices. Nineteen patients (12 per cent) were partially weaned requiring mechanical assistance of some kind for more than 30 minutes but less than 24 hours a day. Six patients (4 per cent) failed to become free of mechanical assistance for more than 30 minutes a day.

### CONCLUSION

1 Under favorable hospital conditions we may expect an over all 2 year case fatality rate approximating 15 per cent. Eighty five per cent may be expected to survive for a period of more than 2 years.

2 Approximately 85 per cent of those who do survive the acute phase will become independent of all respiratory apparatus within 2 years. The great majority will achieve this goal within 6 months after the onset of illness.

3 At the end of 2 years approximately 15 per cent of the survivors will still require some

form of mechanical respiratory assistance for some portion of each 24 hour period. Very little improvement can be expected beyond the 2 year period. In fact a mortality rate beyond that of normal persons can be expected in these patients. Regarding permanent use of respiratory equipment it is known to the National Foundation for Infantile Paralysis that there are in the United States 1700 patients who use mechanical respiratory aids for at least part of each day.

4 Finally what is the outlook for poliomyelitis patients with minimal and mild loss of respiratory function? Clearly this must be much better than the statistics which I have just quoted. Exact data on this point are not available.

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# *Statistical Considerations of Mortality and Prognosis of Patients with Respiratory Disturbances*

DR W RITCHIE RUSSELL

Twenty years ago the case fatality rate in some large outbreaks of poliomyelitis was more than 20 per cent of all paralytic cases and clinicians will agree that the cause of death in the great majority of the cases listed must have been some form of respiratory insufficiency.

The methods of treatment developed in the past 20 years have changed this picture completely so that the paralytic case fatality rate should now in general be below 5 per 100 paralytic cases and in some large outbreaks has been brought down to a much lower figure. Thus I think it should be mentioned that in Los Angeles County California in 1954 to 1955 there were over 1 000 paralytic cases of which 175 required respiratory aids or tracheotomy and yet only 15 died—a paralytic case fatality rate of less than 1.4 per cent. This is surely a record and one to be proud of!

This improvement in mortality began first in the United States in relation to the interest forced on the medical profession by the great epidemics in the 1930's. In some respects the methods developed in the United States for saving life in acute poliomyelitis have until recently remained far ahead of those used in other countries of the world thanks partly to the remarkable assistance in research provided by the National Foundation and partly to the disorganization of research in Europe brought about by the second great war.

To some extent statistics now can be used to study these changes owing to the surprising fact that during the past 10 years in both the United States and Britain the case fatality rate seems to have been almost entirely uninfluenced by the type and severity of the epidemics that have occurred.

Twenty years ago the case fatality rate varied considerably from year to year but it seems that modern methods of saving life have abolished this variation to a large extent as there is no

evidence to suggest that the disease has become less severe. On the contrary these figures suggest that this proportion of life threatening cases remains fairly constant from one outbreak to another and as in the great epidemic in Denmark of 1957 one quarter to one third of all paralytic cases are life threatening in large outbreaks of the disease—I think perhaps in all countries of the world. I think the idea that the stability of the outbreaks changes much from one outbreak to another is countered by the statistics of the last 10 years.

The steadily declining level in case fatality rates is simply an indication of improved methods of saving life during the acute stage. If this is so then we may conclude that the best and easiest indication of the standard of treatment in any one outbreak is provided simply by the case fatality rate for the paralytic cases in that outbreak.

Only in recent years have suitable statistics become available for such studies and indeed the figures for England and Wales are the only ones that I have seen in which there is both an adequate separation of paralytic from nonparalytic cases and at the same time sufficient cases in each year for analysis and comparison of one year with the next. The Danish reports separate paralytic cases but in many years they have listed few patients. Some of the United States reports separate paralytic cases from nonparalytic ones but there is a large unspecified category so that for the whole country the paralytic case fatality rate can be assessed only approximately by assuming that the proportion of paralytic to nonparalytic cases in designated cases remains the same in those which are reported as unspecified. This calculation has been incorporated in Table 160 and the figures from which this is prepared are published in the *Excerpta Medica* with which we all have been provided.

TABLE 160 NOTIFICATIONS AND CASE FATALITY RATES FROM 1945 TO 1956 FOR ENGLAND AND WALES (E &amp; W) AND UNITED STATES (U.S.A.)

POLIOMYELITIS		1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956
Total notifications per 100 000	F & W	20	26	18.1	43	137	176	59	89	100	43	140	65
	U.S.A.	10.3	18.4	7.6	19.0	78.3	27.0	18.5	37.2	22.5	23.9	17.6	9.2
Case fatality rate per 100 total notifications	F & W	16.2	18.8	9.1	13.0	11.0	9.7	8.3	7.6	7.4	6.8	4.3	7.4
	U.S.A.	8.7	7.2	5.4	6.8	6.6	5.7	5.5	5.4	4.1	3.6	3.6	3.9†
Paralytic cases per 100 000	E & W						12.5	3.4	6.1	6.6	7.9	8.2	4.5
	U.S.A.						11.9	23.3	17.7	13.8	9.2	4.9	
	(approx.)												
Paralytic case fatality rate per 100 paralytic cases	E & W						13.6	14.2	10.7	11.4	10.2	7.3	5.0
	U.S.A.						8.6	8.6	7.3	6.2	6.9	7.3	

The figures for paralytic cases in USA are only a very approximate they are inflated in the assumption that the proportion of paralytic to non-paralytic cases remains unchanged in the notification data.

D—The paralytic case fatality rate in the great D. N. H. epidemic of 1957 was 10.7—the same figure for England and Wales for that year.

† This approximate figure is based on 10 per cent sample of death certificate.

Table 160 shows the notification of all cases in the United States and in England and Wales during the last 11 years and the fatality rate per 100 cases. It is quite clear that the great variation in the severity of the epidemics in both the United States and England and Wales

has had no appreciable effect on the steady reduction of the case fatality rate during these years.

In the right part of the diagram (Fig. 299) is the same information recorded with regard to paralytic cases. In England and Wales the re-

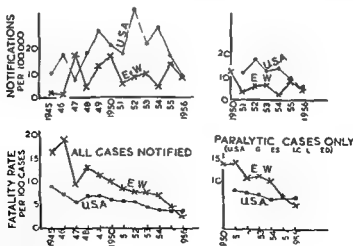


FIG. 299 Fatality rate diagrams indicating all cases notified in England and Wales and the United States the notifications per 100 000 and the rate for paralytic cases only.

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## Statistical Considerations of Mortality and Prognosis of Patients with Respiratory Disturbances

DR. W. RITCHIE RUSSELL

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This improvement in mortality began first in the United States in relation to the interest forced on the medical profession by the great epidemics in the 1930's. In some respects, the methods developed in the United States for saving life in acute poliomyelitis have until recently remained far ahead of those used in other countries of the world, thanks partly to the remarkable assistance in research provided by the National Foundation and partly to the disorganization of research in Europe brought about by the second great war.

To some extent, statistics now can be used to study these changes, owing to the surprising fact that during the past 10 years, in both the United States and Britain, the case fatality rate seems to have been almost entirely unaltered by the type and severity of the epidemics that have occurred.

Twenty years ago the case fatality rate varied considerably from year to year, but it seems that modern methods of saving life have abolished this variation to a large extent, as there is no

evidence to suggest that the disease has become less severe. On the contrary these figures suggest that this proportion of life-threatening cases remains fairly constant from one outbreak to another and as in the great epidemic in Denmark of 1952, one quarter to one third of all paralytic cases are life-threatening in large outbreaks of the disease—I think perhaps in all countries of the world. I think the idea that the stability of the outbreaks changes much from one outbreak to another is colored by the statistics of the last 10 years.

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TABLE 160 NOTIFICATIONS AND CASE FATALITY RATES FROM 1945 TO 1956 FOR ENGLAND AND WALES (E &amp; W) AND UNITED STATES (USA)

POLIOMYELITIS		1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956
Total notifications per 100 000	E & W	2.0	2.6	18.1	4.3	15.7	17.6	5.9	8.9	10.0	4.3	14.0	8.5
	U S A	10.3	18.4	7.6	19.0	28.3	22.0	18.5	37.2	27.5	23.9	17.6	9.2
Case fatality rate per 100 total notifications	E & W	16.2	18.8	9.1	13.0	11.0	9.7	8.3	7.6	7.4	6.8	4.3	2.4
	U S A	8.7	7.2	5.4	6.8	6.6	5.7	5.5	5.4	4.1	3.6	3.6	3.9†
Paralytic cases per 100 000	E & W						17.5	3.4	6.1	6.6	2.9	8.2	4.5
	U S A (approx)						11.9	23.3	12.7	13.8	9.2	4.9	
Paralytic case fatality rate per 100 paralytic cases	E & W						13.6	14.2	10.7	11.4	10.2	7.3	5.0
	U S A						8.6	8.6	7.3	6.7	6.9	7.3	

The figures for paralytic cases in USA are only very approximate; they are calculated on the assumption that the proportion of paralytic to total cases remains unchanged in the unepidemic notifications.

D—The paralytic case fatality rate in the great Danish epidemic of 1952 was 10.7—the same figure as for England and Wales for that year.

† The approximate figure based on a 10 per cent sample of death certificates.

Table 160 shows the notification of all cases in the United States and in England and Wales during the last 11 years and the fatality rate per 100 cases. It is quite clear that the great variation in the severity of the epidemics in both the United States and England and Wales

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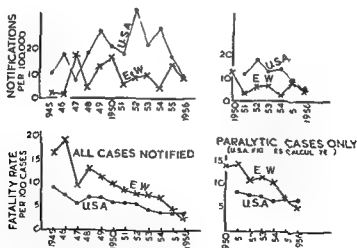


FIG. 299 Fatality rate diagrams indicating all cases notified in England and Wales and the United States: the notifications per 100 000 and the rate for paralytic cases only.

liable recording of the paralytic cases began for the first time in 1930 and about the same time figures appeared in the United States but these have had to be calculated with the assumption which I have indicated so that they may not be clearly reliable. But the figures for England and Wales are entirely reliable and the very steady reduction of the case fatality rate is a very striking feature. In the Danish outbreak in 1952 the paralytic case fatality rate was 10.7 and it is interesting that the paralytic case fatality rate in England and Wales for that year was exactly the same—10.7.

As the proportion of nonparalytic cases reported varies greatly from year to year for a variety of irrelevant reasons and as the diagnosis is in any case usually wrong it would seem that the *paralytic case fatality rate* is the most reliable guide with regard to the quality of treatment and yet the *total case fatality rate* for the earlier years is not without its interest.

The steady decline in fatality rate shown in the foregoing Figure 299 is most gratifying and gives a vivid indication of the great advances in technic required to save life. As far as Britain is concerned the research for this subject began intensively about 10 years later than it began in the United States and it was initiated by our great epidemic in 1947. The accelerated improvement of the past 2 years in England and Wales probably reflects the great interest and activity which followed the Danish outbreak of 1952. Dr Lassen's modes have not been approved everywhere but his provocative presen-

tation acted as a great stimulus in Britain for which we are grateful. Now we all use his methods for the combined bulbar and respiratory case and we now have plenty of reliable apparatus to maintain it for long periods. The figures for England and Wales now rank with the best in the world and reflect great credit on both the organization now in operation by which life threatening cases are transferred to special centres and the physicians in charge of these hospitals many of who are here who have carried out the work involved.

I am not one of those who approves of any form of nationalism in either science or medicine but perhaps it would be a good thing if there were some health rivalry with regard to getting these figures down in various countries. In any case we in Britain may have a severe challenge ourselves this year as already the number of cases is running high and I am sure we will have your sympathy in our attempts to improve still further on our paralytic case fatality rate.

We all are looking forward to the disappearance of paralytic poliomyelitis and it is gratifying to know that the methods of artificial respiration and other methods which have been developed for poliomyelitis are proving now to be of great value in saving life in other diseases.

Finally sir I am greatly indebted to Dr Bradley, Dr Nathanson and Dr Landauer for providing figures on which these comments have been based but I rather believe that they would like me to add that they share no responsibility for interpretation.

# *A The Clinical Diagnosis and Evaluation of Respiratory Problems in Patients with Acute Poliomyelitis*

DR BJORN IBSEN

Justification for repetition in the presentation of fundamental subjects can be found in a change in approach as a result of recent developments in the treatment of patients with respiratory insufficiency. This results in changes in the evaluation of what is considered important with a bearing on indications for treatment.

Five years ago in Copenhagen during the big poliomyelitis epidemic the main problems in the clinical evaluation were in cases with extreme respiratory insufficiency. The technique of administering artificial respiration was so undeveloped that naturally there was hesitation in submitting patients to this active and dangerous treatment if they had a chance to survive on their own. With this in mind we postponed active treatment as long as possible with the consequence that when it was started there were already many of what we now consider late respiratory complications.

I was allowed to make a demonstration on an extremely ill patient, a 17 year-old girl who had paralysis of all four extremities, atelectasis of the left lung and a temperature of 104°. She was cyanotic and sweating, drowning in her own secretions. A tracheostomy was done immediately under local anesthesia with insertion of a cuffed endotracheal tube. Endobronchial suction was done and the respiration controlled with the help of an oximeter of the Millikan type and Drinkmann's carbosensor. It was easy to demonstrate the facts well known to anesthetists that with underventilation with 100 per cent oxygen it is possible to keep a normal oxygen saturation in the arterial blood and to have a CO<sub>2</sub> accumulation with its clinical signs of rise in blood pressure, sweating, restlessness and so on. This was the explanation for the condition of patients with underventilation in the respirators during oxygen therapy. A parallel

could thus be drawn from Beecher and Murray's work about CO<sub>2</sub> retention during anesthesia. The atelectasis could be treated, the airways protected and the circulatory problems handled.

A more recent patient—who was not a poliomyelitis patient—was a 52 year-old man who was brought in after a traffic accident with a fracture of the skull, 10 fractured ribs on the left side and a rupture of the left lung with pressure pneumothorax and subcutaneous emphysema. Furthermore there was a rupture of the spleen. It was felt that this man would not be able to maintain sufficient spontaneous respiration after the removal of the spleen and that it would be too risky to let him try so we did a tracheostomy and gave d-tubocurarine for 4 days and administered artificial respiration. Then we stabilized the chest wall by traction and gave an intermittent block of the intercostal nerves. On the 10th day the patient's spontaneous respiration was adequate and roentgenograms showed absolutely no changes in the lungs. Unfortunately this man died on the 14th day from a big pulmonary embolism on his second day out of bed. Other colleagues have had similar but more fortunate cases.

This case shows how much confidence we now can have in our active treatment as far as lung complications are concerned and demonstrates what consequences this will have in determining our indications for treatment. What I am aiming at is this—that the interest in the problems of clinical diagnosis and evaluation of respiratory problems has focused on the early signs where only a few years ago at least in Denmark we were more concerned about the late signs.

The more one puts weight on early signs the more one will be dependent on clinical diagnosis and clinical evaluation. That means in the old

Hippocratic way what can be seen heard or felt since this still is the main guide in spite of any improvement in lung function studies laboratory estimations x ray diagnosis and so on

To me the first clinical evaluation concerning respiratory problems in patients with poliomyelitis seems to deal with the criteria for the selection of patients in whom respiratory complications if not existing at present might be expected to develop and to give the indications for transfer of the patients to an intensive therapy unit or a special observation department where every means for observation and treatment shall be available including personnel as well as equipment

During the Copenhagen epidemic in 1952 we used the following as criteria for transfer (1) patients with marked cerebral involvement (2) patients with paralysis in the upper extremities (3) patients having difficulty in lifting the head (4) patients with rapidly progressing paralysis in the lower extremities (5) patients having difficulty in swallowing and (6) patients with paralytic cough

In 1952 we received 345 patients in this category

Two types of patients will arrive in the intensive therapy unit

1 The first type arrives at any stage of respiratory insufficiency. They need immediate evaluation and immediate treatment

2 The other type arrives in time and immediate treatment does not seem indicated but eventual progression of symptoms must be followed in order to evaluate the right time for active interference

The observation must be intensive and a record should be kept to make it possible to follow variations in the temperature the pulse the blood pressure and the respiration since the clinical evaluation often must be based on changes more than on absolute levels

About the early signs I think that James Wilson has thrown light on their importance. I do not expect to be able to say it in a better way so I shall quote him

The early symptoms of inspiratory involvement result from increased respiratory effort rather than from acute respiratory failure. Many attempts have been made to use elaborate laboratory methods for the early detection of respiratory insufficiency specifically the deter-

mination of hemoglobin saturation levels and of blood carbon dioxide levels. However in spite of respiratory muscle weakness increased effort in breathing may be successful in maintaining normal or even increased alveolar ventilation. Therefore neither of these determinations nor any other blood chemical measurements now available are likely to prove useful in detecting early respiratory weakness and in fact may be misleading. Determination of the reduction of vital capacity is helpful but in the absence of predetermined levels of the patient's normal capacity early and moderate reductions may not be detectable

Since Dickinson who works with Wilson is to be a discussant I shall not deal in detail with this very important matter

The most common classification of more marked respiratory disturbances has differentiated 3 categories (1) paralysis of the primary muscles of respiration (2) pharyngeal and laryngeal paralysis and (3) pathological processes in the medullary respiratory centers

Although this classification is very useful it should be pointed out that mixtures of types in one patient are very common

What can be seen from this? The patient's respiratory state can be judged from his general appearance in terms of behavior and of skin signs as well as from specific respiratory activity. The psychic signs to look for are anxiety restlessness fretfulness and emotional lability. Pallor flushing cyanosis and sweating also can be looked for

Of respiratory action itself the frequency depth and rhythm should be evaluated. One should look for use of accessory muscles flaring of the nostrils diaphragmatic or thoracic breathing and asymmetry of the movement of the thorax especially paradoxical respiration

In anesthesia I consider paradoxical respiration as a clinical sign which never should be allowed to develop. However if it does it requires immediate active treatment. In paradoxical respiration the ribs are drawn in during inspiration. It may be symmetrical on both sides or more or less localized. The causes can be classified as paralysis obstruction and atelectasis. Sometimes they are more or less combined. The clinical evaluation is very important since it has a bearing on the treatment

What can be heard? Respiration should be no secret. When obstruction of the upper air

ways occurs increased effort is needed to draw the necessary amount of air through the narrowed passages giving rise to increased speed at the narrow place and thus more friction and increased noise. This happens if the patient has sufficient muscle power to overcome the increased respiratory work. This noise can be localized thus giving information about the site of the obstruction. If the patient does not have sufficient muscle power—which very often is the case in polio—the noise is not an absolute indication of the degree of obstruction. Sometimes the vital capacity can be estimated too if one asks the patient to blow to whistle or to count from 1 upward.

ask the patient to cough. This cough can be paralytic or nonparalytic. When the patient is coughing one can hear whether the cough is wet or dry. This seems to me a main point in the evaluation of the kind of further management required. Roughly speaking the outline can be as follows:

1 If the cough is dry and nonparalytic there is time for observation.

2 If the cough is dry and paralytic there might be indication for assisted respiration in a tank or cuirass.

3 If the cough is wet and nonparalytic there is indication for a trial of postural drainage with possibly some suction and physiotherapy before the necessary tracheostomy is done.

4 If the cough is wet and paralytic there is indication for tracheostomy and immediate treatment with controlled respiration in some way or another.

What can be felt? The temperature and moistness of the skin can be felt. The secretions in the bronchi can be felt easily and sometimes can be localized better this way than with a stethoscope. The paralysis in the extremities can be felt and followed. The pressure of the patient's forced expiration is a good clinical guide in determining the vital capacity.

## CONCLUSION

The severe polio cases present themselves with such wide variations in and complexities of symptoms that it is impossible to give definite rules for evaluation and treatment. Each case has to be considered individually. But it is

worth remembering that nearly every case has parallels in clinical conditions familiar to any anesthetist whose job it is to watch and protect the vital functions sometimes under the most difficult conditions. Since I am an anesthesiologist myself I hope you will excuse the fact that I look at the polio cases from an anesthesiologist's point of view.

What parallels can be drawn? First there is the unconscious patient who cannot maintain a clear airway. Such a patient is similar to one in the poisoning center who has had too much morphine or barbiturate or to a patient with cerebral trauma or an intracranial vascular accident. He is similar also from a respiratory point of view to the postanesthetic patient in the recovery room. The unconscious patient cannot protect his own airways—postural drainage to avoid aspiration is essential and depression of respiration might call for stimulation or assisted ventilation. The patient might be in shock and need fluid therapy along the usual lines.

The patient with swallowing difficulties is known to the anesthetist in another way. Patients with local anesthesia in the throat after esophagoscopy or bronchoscopy must be protected against aspiration due to a lack of normal pharyngeal and laryngeal reflexes. Patients with Wallenberg's syndrome in whom the 9th and the 10th cranial nerves are paralyzed are treated along the same lines.

General paralysis may be due to extreme curarization after anesthesia or severe tetanus cases may have been converted by curarization into a resemblance to polio cases. Myasthenia gravis patients in crisis or patients with a severe lack of potassium are both familiar to the anesthetist and are treated with his help. Some polio patients are similar. Polio cases with progressive paralysis of the lower extremities show the same problems as patients with high spinal anesthesia or patients with Guillain Barre syndrome who are treated by anesthetists.

The anesthetist has to deal with patients with all types of pulmonary complications before during and after anesthesia. He has to treat them and follow them and the same principles will be used in polio lung complications. Polio patients with ileus and with depressed respiration due to high lying diaphragms are similar to patients with ileus in the surgical



clinic in the potential danger of vomiting and aspiration resulting in interference with function of the lower lobes of the lungs forming compression atelectasis

The anesthetist is used to handling patients with tracheostomies anesthetizing them and co-operating in physiotherapy inhalation therapy and control of the conditions of the air around the tracheostomy

Poliomyelitis has done much to improve understanding of the proper management of patients with respiratory insufficiency. This management and the clinical evaluation in polio does not consist of special problems but only of problems similar to the ones which are always found where respiration is impaired to a small or a high degree finishing with absolute respiratory insufficiency. An attempt should be made to combine the treatment of all conditions of respiratory insufficiency along the same lines which have been developed so wonderfully through the vast experiences gained in polio work. When polio incidence goes down let us use the co-operation of anesthetists who can form a pool of trained personnel and the respirator centers for treatment of any respiratory

insufficiency. As long as a patient cannot breathe sufficiently it does not matter much why since the immediate therapeutic challenge is overwhelming and the same in all cases to administer a sufficient exchange of air in one way or another. When this has been done it can then be evaluated which symptoms were due to the disease itself and which were due to inadequate respiration. The figures from the result of active treatment are so encouraging that the conclusion can be drawn that many of the symptoms previously thought to be due to the disease were due to improper management of the respiratory problems. When adequate respiration is secured early with specialized technic we will not see many of the late signs of respiratory insufficiency.

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## DISCUSSION

Dr. DICKINSON I have the utmost respect for Dr. Ibsen and for the contributions he and his colleagues have made toward better medical care in poliomyelitis. I have been unable to find any important arguments with the basic concepts presented in his paper and perhaps my time can be utilized most effectively by accepting Dr. Ibsen's invitation to discuss in more detail the early signs of respiratory involvement in poliomyelitis.

Dr. Ibsen has delineated the major categories of respiratory difficulty occurring in the acute phase of this disease namely (1) paralysis of the respiratory muscles (2) airway obstruction from laryngeal or pharyngeal paralysis and (3) malfunction of the medullary regulating centers.

Respiratory difficulty in a poliomyelitis patient can result from involvement in one or any combination of the basic areas just mentioned. However separate discussion of these problems is advantageous.

First let us discuss the progression of signs and symptoms resulting from progressive paralysis of the muscles of respiration. The respiratory apparatus is designed to supply adequately metabolic needs during periods of violent exercise and consequently the normal person can produce a degree of pulmonary ventilation from 15 to 20 times as great as he needs in the resting state. It is logical then to assume that extensive paralysis of the respiratory muscles must occur before one should expect respiratory failure to result and in the vast majority of cases this is true. However signs of respiratory difficulty appear long before the point of respiratory failure has been reached. Respiration in the resting person is normally carried out with little or no visible effort.

This tremendous reserve in ventilation capacity makes effortless breathing possible. As more and more neuromuscular units are rendered inactive by progressive acute poliomyelitis the remaining muscle fibers assume an ever increasing load until fatigue ensues. In an effort to rest his fatigued respiratory muscles the polio patient begins to use the accessory muscles of

respiration and as Drs. Wilson, Ibsen and others have emphasized a combination of decreased respiratory reserve, fatigue and increased effort is responsible for the early clinical signs of respiratory difficulty or dyspnea in the acute polio patient with respiratory paralysis. Oxygen lack and/or carbon dioxide retention may not occur until respiratory muscle paralysis has become extensive and the clinical picture of dyspnea is clearly evident. In fact we have shown that in many patients with early and partial respiratory muscle paralysis actual hyperpnea occurs in spite of this handicap.

The specific signs and symptoms related to respiratory difficulty from paralysis at the respiratory muscles can be grouped into three categories in the general order of their appearance. The first group includes those signs and symptoms related to decreased respiratory reserve, fatigue and increased effort but not related in any direct way to actual respiratory failure. These include weak cough, interrupted staccato speech, increased rate of breathing, increased heart rate, dilatation of the nostrils and use of the accessory respiratory muscles. The next group includes those which may or may not be caused by actual respiratory failure. In this group we find anxiety, restlessness, sleeplessness and disorientation. A third group includes those signs and symptoms of respiratory failure. Here we find cyanosis, hypertension, convulsions and coma. Some of these manifestations are self explanatory and every physician easily recognizes them; some need further discussion.

**Weak Cough.** The normal cough requires a weak breath several times the size of the average resting tidal breath and the weak cough associated with respiratory muscle paralysis is due to the patient's inability to take a deep breath and also may be due to selective paralysis of the abdominal muscles with a resultant lack of ability to exhale forcibly. The weak cough of pharyngeal paralysis is due to the patient's inability to close off his airway at the level of the glottis. This patient in effect blows a rather than coughs. The weak cough of respiratory muscle paralysis is in general normal in character except

for marked decrease in intensity. As the vital capacity falls one would expect the cough to become progressively weaker.

**Interrupted Speech.** This is a useful sign of respiratory muscle paralysis and is characteristic of a sharp diminution in vital capacity. Because of this diminution a patient even before he is quite aware of his own deficiency may talk in a manner in which the use of only a few syllables is interrupted by an inspiratory effort. This obviously results from his inability to take a deep breath. Adults on being engaged in an ordinary conversation may thus well demonstrate the reduction of vital capacity. In children we have to resort to other technics. It is useful to induce older children to count and to ask them how many numbers they can count in just one breath. With the co-operation which is so frequent in patients with poliomyelitis and which distinguishes this condition from many other encephalopathies we are apt to get a useful response to our request. Refusal to speak at all or to use only simple words such as yes, no and water may clearly indicate severe respiratory deficiency.

**Increased Rate of Breathing.** This sign may be due to worry or fear or to many other conditions besides beginning respiratory muscle paralysis which is in fact one of the rarest causes. The rate of respiration is unstable in most young children and in many apprehensive ill adults as well. Seldom is this sign useful in evaluating early respiratory muscle paralysis.

**Dilatation of the Nostrils.** This is a sign which also is seen in a variety of conditions having nothing to do with poliomyelitis or even with such respiratory difficulty as rapid breathing. One commonly may see both dilatation of the nostrils and an increased rate of breathing in children with emotional disturbances or who may have been crying recently. Fever alone may cause this sign of dyspnea to appear. In older children in adults and in young children who are otherwise stable the appearance of this sign however is presumptive evidence that respiratory muscle paralysis or fatigue is becoming progressively more severe.

**Use of Accessory Muscles of Respiration.** The use of the accessory respiratory muscles such as the platysma and sternocleidomastoid is

such striking evidence of respiratory difficulty that it might well be excluded from a list of signs and symptoms of early respiratory weakness. However demonstration of the use of these accessory muscles can be brought about by certain procedures when such use is not spontaneously evident. By inhibiting the action of the diaphragm by splinting the belly with one's hands one can exaggerate the difficulty owing to intercostal weakness and can bring about the use of accessory muscles or dilatation of the nostrils or other evidences of mild dyspnea. Likewise in case of diaphragmatic weakness splinting of the thorax with one's hands can bring about similar evidences of dyspnea not previously apparent.

**Anxiety.** Anxiety can be seen in any hospitalized child or adult. However as evidence of air hunger or of a feeling of inadequacy of pulmonary ventilation it is very important. One can evaluate the symptom only in light of the whole situation and of an understanding of the patient. Its persistence with reassurance with the comfort of the presence of the parents and with the absence of doctors and their needles may be clear evidence of respiratory difficulty or even respiratory failure although it may still be due to other conditions.

**Restlessness.** This may be due either to hypoxia or to carbon dioxide retention. However restlessness combined with sleeplessness may occur in the absence of actual respiratory failure being rather a result of a combination of tremendous fatigue and the need to keep awake in order to breathe. We find that broken momentary sleeping episodes which alternate with wakefulness are an ominous sign of respiratory difficulty.

**Disorientation.** This also may be due to hypoxia or to carbon dioxide retention. This sign of course can result from direct invasion of the brain by the virus or from cerebral inflammation directly related to the infection but having nothing whatever to do with the state of respiration. However it is probably the result of respiratory failure far more often than is generally recognized. One too easily concludes that the virus which attacks the central nervous system has by direct action caused disorientation and mental confusion and fails to recognize

that these symptoms may be a secondary by product of respiratory failure

Cyanosis. Blueness is a traditional sign of hypoxia and one that is easily understood. Little further comment is needed here except to point out that it is indeed a very late sign. When cyanosis occurs with respiratory difficulty due to paralysis one can be sure that the end is not far off. With the blue cyanosis of actual respiratory failure there should be no need for chemical determinations to demonstrate such failure. Cyanosis in poliomyelitis is of course not necessarily the result of muscle weakness alone. One expects any deficiency in carbon dioxide excretion and in oxygen supply to be parallel. This is not always true. We may find oxygen lack to the point of cyanosis without accompanying carbon dioxide retention. Such a situation is not produced by underventilation alone and clearly indicates disease within the lungs themselves such as pneumonia or atelectasis. Patients who develop nearly complete obstruction of a large bronchus usually because of aspiration of mucus or saliva may suffer from oxygen lack without carbon dioxide retention. Perfusion of this obstructed segment of lung by pulmonary circulation may continue. Obviously hemoglobin in blood passing through this segment of the pulmonary circulation will remain unsaturated. This results in a net unsaturation of hemoglobin which if it becomes sufficiently severe will result in cyanosis. However carbon dioxide retention may not occur in this situation since such retention in the obstructed area can be completely compensated for by increased excretion of carbon dioxide in the remaining lung tissue which is well ventilated. In some cases the disturbance in the balance of these two gases cannot be explained by either of the mechanisms mentioned.

Convulsions and Coma. These are agonal and terminal evidences of hypoxia and do not need further discussion.

We do not wish to imply through emphasis upon clinical signs and symptoms that laboratory procedures cannot be of great help in evaluating the patient with progressive respiratory paralysis. Although biochemical changes of failure do not occur early nevertheless carbon dioxide tension and oxygen saturation studies

can be useful later. These chemical studies are also important for proper management once respirator aid has been instituted.

The most helpful laboratory test during early respiratory muscle paralysis is serial vital capacity determinations. Clinical symptoms of decreased reserve rarely appear before the vital capacity has dropped to somewhere between 75 per cent and 50 per cent of normal. A decrease of this magnitude is easily recognized and an exact knowledge of the normal vital capacity for each patient is not essential. In children the test can be particularly difficult since the use of a face mask or mouthpiece in an apprehensive child may so disturb him that co-operation becomes impossible. Nevertheless after two or three trials most patients even though very ill will co-operate with the procedure without being disturbed and a progressive fall in vital capacity can be accurately followed.

The practical question always arises. When is the correct time for the initiation of treatment of a patient in a respirator? This cannot be answered in any certain and absolute way on the basis of any measurement which we can make such as with the spirometer nor can we give an absolute answer by clinical observation alone since emotional factors enter into the decision. Generally the use of a mechanical respirator is indicated as soon as the paralysis has progressed to the point where the patient has developed dyspnea and is becoming fatigued from the effort of breathing. There are two reasons for this: (1) there is good indirect evidence that if a muscle is exercised to the point of exhaustion during acute poliomyelitis the ultimate recovery of that muscle is jeopardized (this concept as logically applies to the respiratory muscles as to the extremity muscles) and (2) it is important to institute mechanical aid or oxygen lack may further damage nerve cells invaded by the virus.

Therefore we do not look upon the respirator solely as a device to prevent death from respiratory failure and we should initiate its use long before extreme dyspnea or clinical or laboratory evidences of respiratory failure actually occur. The machine should be used at the time when respiratory muscle paralysis is first detected. At

this time some evidence of dyspnea can be detected even if it is made evident only by inhibition of the action of the diaphragm or of the intercostals as described previously. Only actual trial can determine in many cases how successful a respirator will be in producing rest

I would agree with Dr Ibsen that the early use of a mechanical respirator before the patient has become completely exhausted may well drastically reduce the incidence of severe pulmonary complications in patients with respiratory muscle paralysis.

# *Control of Respiration*

## *B. Techniques for Providing Artificial Respiration The Tank*

DR. ROBERT M. EIBEN

The tank respirator undoubtedly has been the most extensively employed mechanical device to provide artificial respiration for patients with prolonged respiratory muscle paralysis. It apparently has long been recognized that cyclic changes of pressure around the body result in passive respiration. This principle which ultimately resulted in the development of the tank respirator was suggested or applied in the management of patients with respiratory problems long before the present-day respirator was designed.

The first tank respirator used in the management of patients with respiratory poliomyelitis was developed by Drinker primarily as a laboratory device. Intertank pressure changes were accomplished by an electrically powered centrifugal pump. The range of pressures obtainable with this instrument considerably exceeded that obtainable in the modern version which is operated by a motor driven bellows. Few of the respirators in use in the country at the present time are capable of producing pressures lower than negative 30 centimeters of water and higher than positive 10 centimeters of water.

In an attempt to make the tank respirator more universally applicable in the management of severe respiratory insufficiency positive pressure attachments have been added to the newer models. The positive pressure dome fits over the head end of the respirator and can be engaged only when the tank is opened. The Ben-ner positive pressure attachment permits the synchronous application of positive pressure by means of a face mask or a tracheotomy adapter. This device can be employed to augment the pressures obtained within the tank. Both attachments provide adequate support for the patient when the tank is opened for nursing and physical therapy procedures.

Since its development the tank respirator has been used with some apparent reluctance by all physicians. Although all would acknowledge that the machine is a valuable device there is nor infrequently procrastination before the patient is placed in the machine. Whether this concern is the result of anxiety over possible harmful physiologic effects or merely a manifestation of the basic conservatism of the average physician remains obscure. If we accept the concept that exhaustion and inadequate ventilation are injurious to the poliomyelitis patient it follows that early respiratory support is necessary.

Initiation of artificial respiration in the patient with acute poliomyelitis is usually accomplished as a result of the clinical interpretations of observations made on the patient. Mild respiratory insufficiency may be manifested only by restlessness, inability to sleep or unwillingness on the part of the patient to talk or to be otherwise disturbed. Paradoxical breathing, inability to count slowly to 10 and flaring of the alae nasi are manifestations of severe respiratory distress and are in themselves indications for immediate respiratory aid.

Vital capacity measurements are of value in the demonstration of mild respiratory insufficiency and when followed closely may be used as a guide for the initiation of artificial respiration. I suggest that the patient be introduced to the respirator when the vital capacity has fallen to approximately 50 per cent of predicted normal. This rather arbitrary figure is preferred because preparation of the patient can be accomplished in an unhurried and reassuring manner. If the patient fails to relax or to synchronize his own respiratory effort with the respirator it will be safe to return him to his bed. Such early failure may at times prove indirectly beneficial.

for the fact that the patient could be removed from the machine may decrease his anxiety regarding it and make him somewhat less fearful of subsequent preparation for tank respirator support.

The patient should be placed promptly in the tank respirator if the vital capacity falls to 35 per cent. Support in the respirator should be continuous except for brief periods when the patient's respiratory efforts are observed or when nursing procedures are carried out. In general the cuirass respirator and rocking bed are not recommended for use in the acute illness. The so-called lesser aids do not have the ventilatory efficiency of the tank respirator and requirements of the patient cannot reliably be met with their prolonged use.

Once the patient is established in the tank respirator the major medical responsibility is to ensure the adequacy of the ventilation provided by the machine. No small part of the management of the patient within the tank is the maintenance of an unobstructed airway. The wide range of pressures available within the tank are under ordinary circumstances adequate for most patients. On rare occasions particularly in the tracheotomized patient the intertank pressure must be supplemented by the synchronous application of positive pressure to obtain the desired pressure differential. Generally intertank pressures of negative 15 to 20 centimeters of water and positive 5 centimeters of water will adequately ventilate most adults. Observations that intertank pressures as low as 10 centimeters of water applied to the normal subject will over ventilate him dramatically emphasizes that changes in the elastic qualities of the lung and the thorax of the polio patient do occur.

One cannot depend upon the patient's interpretation of the adequacy of ventilation for if he is alert it is likely that he will demand over ventilation whereas if his sensorium is clouded it is likely that he will be insensitive to air exchange which is totally inadequate. There is no substitute for the actual measurement of tidal ventilation and minute volume. The nomogram of Radford has proven extremely valuable as a guide in providing adequate respiration. In use the recommended tidal ventilations determined are exceeded by approximately 10 to 15 per cent.

When hyperventilation of the patient is recog-

nized the pressure changes to correct this situation must be made slowly. The patient with some remaining respiratory ability may unconsciously assist the respirator. Tidal exchange measured under these circumstances will not obtain when the patient fatigues and if intertank pressure is not increased to compensate for this reduction inadequate ventilation will occur.

The tank respirator cannot prevent the development of pulmonary complications. The loss of an effective cough and postural influences on the drainage of the respiratory tree often result in areas of malfunctioning lung ultimately leading to areas of atelectasis and/or bronchopneumonia. Recognizing this interference with the adequate drainage of the respiratory tree we change the patient's position frequently. Most patients should spend at least several periods of time daily in the prone position in the tank respirator. After a tracheotomy it is no longer possible to turn the patient on his abdomen in the tank respirator and the management of the patient cannot be accomplished entirely satisfactorily unless there is some way to provide positive pressure through the tracheotomy tube when the patient is placed in this position.

The tank respirator interferes with the diagnosis of pulmonary complications because of the dependence of the clinician upon physical diagnostic criteria. However measurements of ventilation often will indicate difficulties in respiration that could not be detected by the experienced clinician and decreased tidal exchange at previously established satisfactory pressures often precede roentgenographic evidence of atelectasis and pneumonic process.

Although there is a significant incidence of pulmonary infection in the respiratory poliomyelitis patient it is not considered desirable to place patients routinely on prophylactic antibiotic therapy simply because they are placed in the tank respirators. When pulmonary infections occur they should be treated vigorously with antibiotics and chemotherapeutic agents and therapy should be discontinued when resolution of the process has been demonstrated.

Technics for intermittent deep breath have been developed and would appear to be of great value in preventing atelectasis. Intermittent deep-breathing attachments can be attached to

individual tank respirators or used with several machines by employing a flanged fitting to exhaust air from the tank through one of the arm ports. A series of deep breaths are accomplished by reducing the pressure within the tank to negative 20 or 40 centimeters of water.

### SUMMARY

The successful use of the tank respirator in the management of the patient with respiratory poliomyelitis depends to some extent on its being used by a group of persons who are aware of the value and limitations of the machine. Artificial ventilation must be initiated early certainly considerably in advance of real respiratory distress. Frequent turning of the patient is necessary to provide adequate drainage of the respiratory tree and to permit more even distribution of air in the lungs. Adequacy of ventilation may be determined by measurement of tidal exchange. Arterial blood studies remain the only certain way to demonstrate effective exchange of gases between the lungs and blood. Respiratory assistance should be continuous during the acute illness and weaning from respiratory aids should be guided by measurements of the patient's respiratory ability.

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## Control of Respiration

### *B Technics for Providing Artificial Respiration Intratracheal Positive-Pressure Respirators*

DR GÖRAN HAGLUND

The history of positive pressure respiration starts at the end of the 9th Century B.C. with the Jewish prophet Elisha as we can read in 2 Kings. The first man on record who experimented with regular intratracheal positive pressure respiration was Andreas Vesalius who made such experiments in the middle of the 16th Century A.D. The first to describe an effective and in principle a modern positive pressure respirator was John Hunter the English surgeon in 1776. In the scientific literature published up to date more than 100 different positive pressure respirators have been described and recommended. About half of them have been presented during the last 10 years.

The purpose of all artificial respiration is to give the patient physiologically adequate respiration. In most cases and situations the central problem is to secure an adequate and effective respiratory minute volume. The alveolar exchange of gases is of course the fundamental of respiration but to secure reliable analyses of the alveolar gases is in our opinion clinically difficult. Our biochemist Jorgen Lehman therefore worked out a routine control method with simultaneous measurement of the respiratory minute volume and a simple analysis of the carbon dioxide content of the mixed expiratory air. Every nurse can perform such a control on the ward in about 5 minutes as she is checking the controls on the respirator and she can do it without disturbing the patient. Professor Lehman's method has spared our patients thousands of artery and vein punctures. Every respirator patient must be controlled with pH analyses etc. but by using Lehman's control method laboratory tests can be kept at a minimum.

The first demand made on an automatic positive pressure respirator is that the operator can adjust the respiratory minute volume exactly to

permit this volume to remain constant. Despite moderate variations in resistance as a result of accumulated mucus in the airways the condition of the lung parenchyma counterpressure from the abdomen and so on a first-class positive pressure respirator will provide the patient with a stable respiratory minute volume.

The respiratory minute volume must not only be a constant volume from minute to minute from hour to hour from day to day but also at the same time an effective respiratory minute volume. If the valve system on the respirator is pressure sensitive to permit the pressure changes in the patient's airways to initiate the changing of the valves from inspiration to expiration and back as is often the case the respiratory minute volume pressed into the patient's airways and lungs will be unchanged even if the resistance in the airways and lung parenchyma increases. But the respirator frequency will increase compensatorily. This means an increase in the ventilation of the dead space and a corresponding decrease in the alveolar ventilation. In other words it means a stable but ineffective respiratory minute volume! The other way round a decrease in resistance will lead to a hyperventilating effect.

For these reasons the automatic changer of the valve system of the positive pressure respirator preferably is separated from the inspiratory system. The valve changer must give a uniform respiratory frequency and have a relative wide variability from 10 to 25 in adults and 35 to 40 or more in infants. A stable frequency is a great comfort to the respirator patient and will spare him and the attendants many unnecessary alarms. A constant respiratory minute volume with a stable respiratory frequency will ensure an effective respiratory treatment. During such treatment the control manometer connected

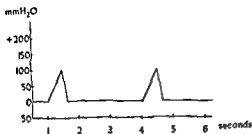


Fig 300 Type curve

with the patient's airways will give the well trained and observant attendant reliable and early clues as to what is happening and it will incite him to proper prevention and treatment of threatening complications in the airways and in the lungs.

The complex mechanism of the venous return and the pulmonary circulation during normal respiration and during positive pressure respiration cannot be discussed in this paper. It is sufficient to say that with our current knowledge we must insist on a pressure curve with a relatively short inspiratory climb to pressure maximum and a still more rapid decrease to the zero line and with a relatively long pressureless pause before the next inspiration (Fig 300). We do not know for sure how to evaluate rationally differences in pressure curves from different respirator models. Four pressure curves from different respirators are shown in Figure 301. I beg your pardon that I don't have the zero line. We see that the pressure curves are quite different. These curves are from a lung model and experiment. One way of comparing different respirators is to measure the pressure masses defined as millimeter water pressure seconds per minute for each respirator during standardized performance tests. Such measurements from 4 Swedish respirators, the 4 of which we saw the pressure curves are shown in Figure 302. We see that there are quite big differences among the pressure masses that these respirators are giving.

Opinions regarding a negative pressure phase after the passive expiration are also controversial. My own clinical experiences indicate that such a suction may be a definite help in some cases with threatening non-ventral circulatory insufficiency. Such a negative pressure is shown in the solid line of Figure 303 as compared with the

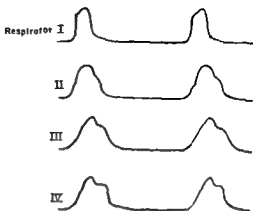


Fig 301 Pressure curves from 4 different respirators

non-negative pressure curve with the dotted line. Such a negative pressure will lower the abscissa of the inspiratory positive pressure and thereby give a decrease of the positive pressure masses. Experiments have shown that the respiratory effect is unaffected by moderate negative pressure phases. We are experimenting further with the theory that a positive pressure respiration with negative pressure expiration where the positive pressure masses are (1) kept minimal for the necessary respiratory effect and (2) exactly counterbalanced by equal negative pressure masses shall give the optimal or the least unphysiologic positive pressure respiration. The effect of the negative pressure of 20 mm H<sub>2</sub>O as illustrated in the figure was to decrease the positive pressure masses from 1750 to 1100 mm water pressure seconds per minute. But the difference between the positive and the negative pressure masses became as low as plus 350 mm water pressure seconds.

In endotracheal respiration most of the natural defense barriers against cold, dry, and dusty air are eliminated. Therefore a positive pressure respirator must deliver efficiently prewarmed, humidified, and filtered air. Tests have shown remarkable differences in these respects between different respirator models. In some cases we have found dangerous defects.

#### SUMMARY

The endotracheal positive pressure respirator should give a constant respiratory minute vol

PRESSURE MASSES				
mm Hg water pressure x seconds per minute				
Minute Volume	liters			
Frequency/minute	16	18	20	
Respirator I	925	660	497	
II	1060	935	640	
III	1100	1015	885	
IV	1490	1345	980	

FIG 302 Differences in pressure masses in 4 different respirators

ume at a stable frequency. The respirator should have a reliable mechanism for shifting of the valve system and for regulation of the different respiratory phases. The respirator should have a device for negative pressure. The air from the apparatus must be efficiently prewarmed, humidified and filtered. The respirator

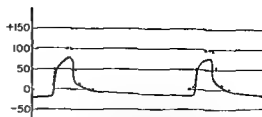


FIG 303 Pressure curves with and without negative expiratory phase

should have a manometer directly connected to the patient's airways.

The technical construction should be simple; all parts—especially the moving parts—should be capable of being easily inspected even when the respirator is in use, and they should be simple to adjust and repair. Unnecessary luxury and ingeniousness does not benefit the treatment nor the budget. In clinical work, lightweight and noiseless respirators are to be preferred. All in all, simplicity and tested reliability is what we need.

## Control of Respiration

### *B Technics for Providing Artificial Respiration Cuirass Respirators, Rocking Beds and Portable Respirators*

DR GEORGE SAXTON, JR

In general cuirass respirators and rocking beds are less effective than the tank respirator or intermittent positive pressure breathing through a tracheotomy tube therefore they are used chiefly during convalescent and chronic stages of poliomyelitis with respiratory paralysis. In other words whenever there are intrapulmonary complications such as increased secretions pneumonitis atelectasis or pneumonia these lesser aids should not be used. However when the lungs are clear most patients can be given sufficient ventilation using cuirass respirators and rocking beds even when respiratory paralysis is complete and the vital capacity zero.

A cuirass respirator can be thought of as an abbreviated tank respirator. It produces passive inspiration by expanding the lower rib cage and drawing the diaphragms toward the abdominal cavity when a subatmospheric pressure is developed in the cuirass by a pump attached by a large hose as illustrated in Figure 304. However in order to be effective this type of device requires a good seal around the edge of the cuirass where it must contact the body. Very little expiratory assistance can be achieved with this apparatus because the seal is broken by positive pressure in the range of 5 to 10 centimeters of water.

The chief advantage of cuirass respirators is that they permit the patient to be free of the tank respirator or the tracheotomy tube. Furthermore with certain shells which do not extend over the pelvis the patient may even sit up. This then permits mobility in a wheelchair or automobile which is impossible in the tank respirator.

However the fitting and application of a cuirass respirator requires some training on the part of the nursing staff and members of the patient's family. In fact most patients with severe scoliosis



FIG 304 Cuirass type respirator

cannot be fitted with a cuirass at all. Also it requires some time for the patient to become accustomed to the cuirass and to tolerate it for 6 to 8 hours at a time. These cuirasses should never be used 24 hours a day because the skin will usually break down under constant pressure.

One of the unique values of the cuirass respirator is that it assists inspiration by producing negative intrapleural pressure and therefore probably aids venous return of blood in the right atrium. However a disadvantage is that a cuirass limits the excursion of the upper ribs



FIG 305 Rocking bed with piston linked to motor



FIG 306 Synchronizing intermittent positive pressure breathing through a tracheotomy tube

during inspiration as can be demonstrated by fluoroscopy. Of course the tidal volume which can be produced by a cuirass respirator is only about two thirds or 60 to 70 per cent of that which can be produced by a tank respirator in a given patient at comparable transpulmonary pressures.

Dr. Eve in England first described the use of the rocking motion in the resuscitation of patients suffering immersion or shock in 1932. The patient was placed on a board over a barrel or some such fulcrum and rocked back and forth. In this way artificial respiration could be produced by the passive motion of the diaphragms resulting from shifting of the abdominal viscera. About 10 years ago Dr. Jessie Wright introduced the idea of mechanizing such a device to give patients with respiratory paralysis artificial respiration over long periods of time. It has been used increasingly since 1930 in the United States so that in recent years many respirator centers have come to use this type of bed for their patients to the exclusion of regular

hospital beds though the rocking mechanism may not be used more than 8 hours in 24. Patients find that they can eat, read or even converse easily while rocking after they have become accustomed to this apparatus.

The chief advantage of the rocking bed is that no equipment needs to be attached to the patient. He need only lie down on the mechanism. However it is the least effective of the various types of respiratory equipment moving even smaller tidal volumes of air in and out of the lungs than the cuirass respirator. Therefore it is particularly well suited for use at night when the metabolic needs of the patient for gas exchange are least and the patient would not have occasion to be elsewhere than in bed anyway.

There are a number of limitations to the rocking bed such as the fact that it produces motion sickness in about 10 to 20 per cent of



FIG 307 Intermittent positive pressure breathing at the mouth



FIG 309 Portable pressure breathing apparatus

patients at first. This almost inevitably clears up with gradually increasing periods of use. Furthermore body configuration is also important in that a patient with a scaphoid abdomen often will not derive sufficient ventilation. A certain abdominal avoirdupois is necessary to serve as a pendulum pushing and pulling on the diaphragms as a result of the rocking motion. Infants and small children do not derive as much ventilation proportionately from rocking mechanisms as do adults.

The rocking motion has a number of side effects in addition to ventilating the lungs. For instance it can help a patient raise tracheo-bronchial secretions in greater quantity than would be expected from the tidal volume produced. However aspiration of secretions from the pharynx is a danger if there is bulbar involvement. Therefore the rocking bed should not be used as a ventilatory aid so long as pharyngeal paralysis persists.

Some think that the rocking motion of such beds will increase cardiac output or at least stimulate cutaneous blood flow. Others hope that the rocking motion might decrease the rate of demineralization of the skeleton which

follows severe paralysis. However these hypotheses remain unproven by measurements.

One of the more interesting developments in the last 5 years has been the combination of the cuirass respirator with the rocking bed. In 1929 Mr. Emerson produced a few experimental models of a rocking bed with a piston linked to the electric motor which rocked the bed as shown in Figure 305. In this way a negative pressure in a thoracoabdominal cuirass could be produced through a hose connected to the piston during the foot-down position of the bed. Tidal volumes produced by this combined action exceeded that produced by either mechanism alone. In practice this combination has proven useful occasionally in weaning patients from the tank respirator or intermittent positive pressure breathing through a tracheotomy tube. It has been used almost exclusively for this transition to lesser aids for a few days or weeks at a time but not usually longer.

Another combination tried more recently consists of synchronizing intermittent positive pressure breathing through a tracheotomy tube with



FIG 309 Portable hand bellows type of breathing apparatus

the foot-down position of the bed (Fig 306). This also is useful chiefly in transition from a major aid to a minor aid i.e. for introducing a patient accustomed to intermittent positive pressure breathing to the rocking motion.

Until this past year most so-called portable respirators have consisted of rather heavy battery operated cuirass respirators. These could be rolled about on a cart or dolly or transported in an automobile but they certainly could not be carried any appreciable distance. However in 1957 a number of companies developed lighter units which can be carried.

Two of these new portable units made by Monaghan Company and Thompson Company use a 15 pound or 25 pound blower instead of a heavy piston to lower the pressure in the cuirass. A somewhat heavier power unit weighing 45 pounds has been developed by the Huxley Company for positive pressure in an inflatable abdominal binder called the Pneumobelt.

Another method of respiratory assistance which is portable has been coming into increasing use over the past few years. It consists of intermittent positive pressure breathing at the mouth achieved by blowing air through a tube which the patient holds between the teeth. In Figure 307 appears a vacuum cleaner which is marketed by the Emerson Company. The patient merely closes his lips around the tube for inflation of his lungs and opens his mouth to permit expiration by elastic recoil of the lungs. In this way the patient determines the depth and frequency of his breathing so that he can



FIG 310 Blacksmith bellows type of breathing apparatus

use it for an occasional deep breath and for lung stretching whenever he likes as well as for tidal ventilation. It can also be used for a weak cough by elastic recoil of the lungs after maximum inflation. Drying of the mouth from the continued blowing during expiration can be prevented by the patient's holding the tip of his tongue over the tube during expiration. Patients often like to breathe with such an apparatus when they are being transferred from one type of equipment to another such as from a tank respirator to a chest respirator or rocking bed. They can use it to good advantage when being transferred to another area of the hospital especially when the purpose is to get a roentgenogram of the chest made or to receive hydrotherapy. Figure 308 shows the lightest portable weighing only 7 pounds which blows air continuously. This is produced by the Thompson

Company and the patient holds the tube in his mouth for intermittent positive pressure breathing. It can run on batteries or alternating current. The ultimate in portable respiratory equipment is the hand bellows (Fig. 309) which is produced by a number of different companies such as the

Emerson Kriesleman and Monaghan Companies and last of all is the old fashioned blacksmiths hearthside bellows (Fig. 310) which can be purchased through the equipment catalogs for only \$5 and with an appropriate valve can do a good job of artificial respiration.



## DISCUSSION

**DR. FERRIS** The importance of an adequate ventilation should be emphasized since it is the primary concern of artificial ventilation. The ventilation should not be too little; the dangers of underventilation with carbon dioxide accumulation and lowered oxygen tension are well recognized. Overventilation is also undesirable since the lowered arterial  $pCO_2$  adversely affects the functioning of the nervous system, produces personality disturbances and makes it more difficult to wean patients from respiratory aids. In our experience the use of the Radford nomogram has been a great asset in estimating the required ventilation. Figure 311 presents a comparison of predicted tidal volumes from the nomogram and those actually measured with simultaneously collected arterial blood. The measured tidal volumes have been corrected to a temperature of 24°C and an arterial  $pCO_2$  of 40 mm Hg, since the nomogram was constructed for such conditions. Here the mean difference is plus 3.04 per cent with a standard deviation of 21.5 per cent; this comparison includes both acute and chronic patients using a variety of respiratory aids or breathing spontaneously. Four of the 5 observations that are most out of line are the ones that are circled in

Figure 311 which were obtained on patients who had known pulmonary disease complicating their poliomyelitis. The nomogram is not suitable in such cases since there may be a low arterial oxygen saturation even in the presence of adequate removal of carbon dioxide. In general the nomogram does permit a good prediction of the proper ventilation. The pressures used in the breathing machines should be set to produce this ventilation. I was pleased to hear that Dr. Haglund and his associates have also used a bloodless method to estimate the adequacy of ventilation of their patients. In general the addition of oxygen to the inspired air will not be necessary unless there is gross pulmonary pathology such as atelectasis or pulmonary edema.

The next point to emphasize is the need to maintain a clear airway. Frequently this can be done merely by the use of the prone or face down position. This allows drainage of secretions from the mouth and minimizes the hazard of aspiration. This position can be used in the body respirator but the required ventilating pressures are higher than they are in the supine position. By this procedure tracheotomy may be avoided. Frequent change of position is equally

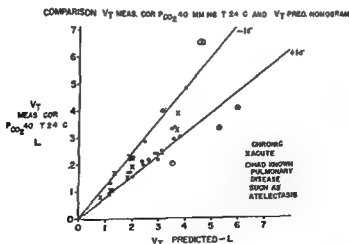


FIG 311 Comparison of predicted tidal volumes with those actually measured

important and must be done regularly whether the patient is in a body or tank respirator or is being respired by one of the other positive pressure breathing devices. This minimizes pressure within the lungs. It also helps to prevent pressure sores on the body.

If a tracheotomy is to be done the largest size of cannula or endotracheal tube that can be inserted should be used to keep the resistance of the airway at a minimum. Any tube that is inserted into the trachea will be smaller than the trachea and the area available for air flow will be still smaller. Because of this its resistance is greater than that of the trachea and more work must be done either by the patient or the apparatus used for breathing to overcome this resistance. When the tracheotomy tube is no longer needed as a route for air exchange smaller tubes should be used. Since the patient is breathing around the tube even smaller tubes can contribute significantly to pulmonary resistance. Figure 312 shows the resistance measurements obtained on a patient with two different sizes of tracheotomy tubes in place and finally with the tube removed. The  $\bar{x}$ s represent the mean value obtained and the lines plus and minus represent two standard deviations from the mean. The differences between the means of each group as tested by student's test demonstrate that the differences are significant at a  $P$  of less than 0.01.

The presence of a No. 3 tracheotomy tube (outside diameter / mm) almost doubles the total pulmonary resistance. This tube is too small to breathe through easily and yet it contributes considerably to the resistance. Measurements on models have shown that this size tube in a tracheal model comparable to this patient's trachea increases the resistance threefold.

Much of our own work in the past few years has been directed toward assessing the efficacy of sizing the lungs and thorax by means of a deep breath. This technique was recommended in the early reports of the tank respirator. It was again suggested during the Minneapolis epidemic of 1946. Both of these techniques used the minimum pressures developed by the tank respirator. Our investigations indicated that these pressures usually about 75 cm H<sub>2</sub>O were insufficient. In order to produce adequate pressures a booster in the form of a vacuum cleaner

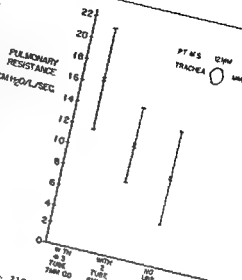


Fig. 317 Effect of tracheotomy tubes on pulmonary resistance

motor was used so that negative tank pressures up to 40 cm H<sub>2</sub>O could be reached. The evaluation of this type of treatment involved measuring the compliance of the lung, in the tidal volume range of patients with poliomyelitis. The effect of one or a series of deep breaths on their pulmonary compliance was studied. For comparison similar studies of the effect of shallow and deep breathing on the pulmonary compliance of normal persons were also made.

The results on a patient are presented in Figure 313. The initial lung compliance on the left is low. After two or more deep breaths in this instance produced by negative tank pressures of 30 to 35 cm H<sub>2</sub>O there is an increase of 20 to 30 per cent in lung compliance. If after a series of such breaths the lung compliance in the tidal volume range is followed serially there is a progressive fall in the lung compliance toward the initial control value. In the patients this appears to take place over a 1 hour to 2 hour period and it is faster when the patient lies in the semiright lateral position as shown to your right.

In a similar study on normal persons the control pulmonary compliance was measured in the supine position as indicated in Figure 314. Following this the subject could be tilted to

means of a tilting bed to a sitting position. Mid position change was prevented by using an inflatable balloon beneath an abdominal binder. With the position change without lung volume change there is an increase in the measured lung compliance. This we believe is due to an artifact introduced by the supine position. However a few deep breaths produced an additional increase in lung compliance. This increase also occurs when the subject lies in the right lateral position and in the sitting position even though lung volume changes are allowed to occur. As

in the patients the lung compliance decreases in these various body positions if the subject continues to breathe in a shallow or limited manner. Here the change is more rapid than that seen in the patient with poliomyelitis.

Because of the prompt reversibility of this phenomenon it is thought that the change in compliance probably is due to parts of the lung closing off and that these areas are reopened by sighing or taking a deep breath.

The patient with poliomyelitis also should stretch his lungs either by means of various

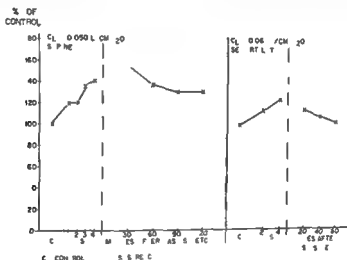


FIG. 313 Per cent change in compliance of lungs as result of deep and shallow breathing in patients

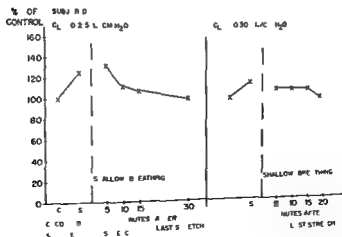


FIG. 314 Per cent change in compliance of lungs as result of deep and shallow breathing in normal persons



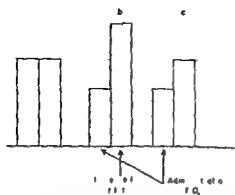


FIG 316 Oxygen-carbon dioxide balance in various pulmonary and cardiac complications in poliomyelitis patients

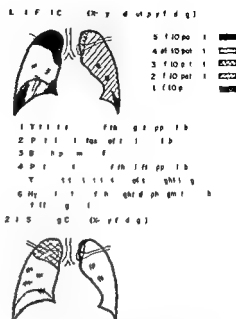


FIG 317 Frequency of pulmonary complications in patients receiving positive pressure artificial respiration

tion. In more severe forms for example when bronchial rules appear we favor tracheotomy and a positive pressure respirator.

Again it is clinical observation which enables us to detect modifications in the O<sub>2</sub>/CO<sub>2</sub> equilibrium and to select the appropriate therapy (Fig 316). The appearance of respiratory insu-

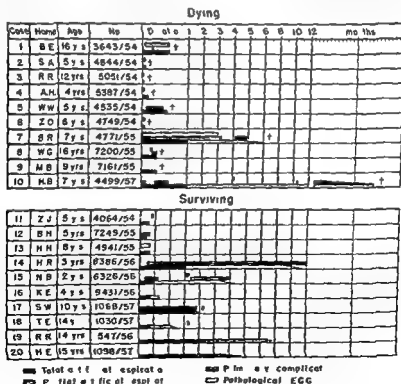


FIG 318 Cases of poliomyelitis receiving positive pressure artificial respiration at the Zurich Kinderspital (Children's Hospital)

iciency as well as obstruction of the airways produce a drop in the oxygen saturation blood level and an accumulation of CO. Administration of oxygen corrects the hypoxia while an increase in ventilation permits the elimination of CO. However the occurrence of pulmonary edema or cardiac insufficiency will require only administration of oxygen.

The choice of the method to be used does not depend solely on the degree of respiratory failure or the state of the respiratory passages. It also depends on the general clinical signs of the illness. In cases where peripheral paralysis is very pronounced requiring intensive physiotherapy and frequent changes of position the positive pressure respirator is indicated because it has undeniable advantages in such cases. The same applies to cases in which hygienic care and control of the course of the disease (pulmonary and cardiac auscultation, repeated electrocardiograms, etc.) are such as to call for a simple approach. (See Table 16<sup>7</sup>.)

TABLE 16<sup>7</sup> INDICATIONS FOR USE OF IRON LUNG AND POSITIVE PRESSURE RESPIRATOR

	IRON LUNG	POSITIVE PRESSURE RESPIRATOR
1. At the onset of respiratory insufficiency	+++	
2. When the respiratory passages are clear	+++	+
3. When the respiratory passages are obstructed by mucous secretions	+	+++
4. Physiotherapy	+	+++
5. Hygienic care	+	+++
6. Control of the course of the disease	+	+++
7. Psychological effect	+	+++

I join Haglund in insisting on the necessity not only of heating, but also thoroughly humidifying the air used. This was achieved by increasing the temperature of the water in the Engstrom apparatus. With us too the tidal volume range was determined with the aid of a Ralston nomogram. Our experience has shown—and this confirms the view of Liben—



FIG. 319 Disturbances of calcium metabolism due to immobilization

that the figures must be increased by 10 to 20 per cent especially in the case of very young children. The problem of antibiotic prophylaxis was solved in our cases by systematic administration of a broad spectrum antibiotic which must be selected and its necessity changed according to the findings of the sensitivity tests (antibiogram). We feel that such prophylaxis must be kept to a minimum and discontinued as soon as possible. However it must be reinstated at the slightest indication of pulmonary complication.

It has been claimed that positive pressure respirators cause atelectasis especially of the upper lobe more frequently than does the iron lung or Drinker respirator (Fig. 317). Various hypotheses have been postulated on the pathogenic mechanism of this complication. If we mention it today it is primarily to emphasize the importance of procedures designed to drain the bronchi, postural drainage and of systematic and repeated clearance of the bronchial tree which is greatly facilitated by the use of positive pressure respirators. It should not be forgotten that pulmonary complications occur not only during the acute phase but also during the subacute and the chronic phases of the disease.

In the chronic phase respiratory failure proper becomes less important and it is not as necessary to keep a close and repeated check on the O<sub>2</sub>/CO<sub>2</sub> equilibrium. On the other hand cardiac repercussions—an idea I am thinking above all

of the pulmonary or right heart—pulmonary complications, psychological hygiene, electrolytic changes and more especially disturbances in calcium metabolism are assuming ever greater importance (Fig. 318).

We could not confirm in our patients the occurrence of pulmonary emphysema as constantly observed by Haglund. I would be interested to find out on the basis of what criteria Haglund formulates a diagnosis of emphysema! According to our experience the appearance of calciuria—an expression of decalcification of

the organism due to inactivity—does not seem to be affected by use of the rocker bed. Any way this point will be the topic of tomorrow's discussion (Fig. 319).

In conclusion we think that the choice of a method of artificial respiration depends less on the theoretical value of the method as such than on the exact clinical evaluation of the case to be treated. During the course of the disease it is frequently advantageous to switch from one method to another depending on the requirements of the moment.

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# Care of Patients Severely Stricken by Poliomyelitis

THURSDAY MORNING, JULY 11, 1957

(This Session Convened in the Aula of the University of Geneva)

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## *Chairman*

DR. ELIAS BENGTSSON

Epidemic Hospital  
Stockholm

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University of Washington  
Seattle

DR. WILLIAM A. SPENCER

Southwestern Poliomyelitis  
Respiratory Center  
Jefferson Davis Hospital  
Houston

DR. INGEMAR JUNGNER

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Hospital for Infectious Diseases  
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Omaha

DR. AVRON Y. SWEET

The Mount Sinai Hospital  
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DR. LEONARD V. WENDLAND

Rancho Los Amigos Hospital  
Hondo, California

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Ullevål Hospital  
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PROF. DR. PETER HOIST

Ullevål Hospital  
Oslo

DR. LEON LEWIS

Lairmont Hospital  
San Leandro, California

DR. T. NEUKIRCH

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University of Geneva  
Geneva



# *Emancipation from the Respirator Following Poliomyelitis*

DR FRED PLUM

Improved treatment of respiratory failure caused by acute poliomyelitis has produced a sharp reduction in the disease's mortality rate. Where once 90 per cent or more of the patients placed in respirators succumbed at least 8 out of 10 will now survive their acute illness and it is rare to lose a patient once the acute stage of the disease is over. Such success in treating the acute phase is gratifying but has created the problem of the rehabilitation of persons with serious permanent limitations in breathing function.

The present communication summarizes experience with a program of emancipation from respiratory aid employed for the 70 patients who have been discharged from our own center in Seattle, Washington.

Nearly all these patients came in tank type respirators. Although cumbersome and often inconvenient the tank provided a greater respiratory reserve than did chest respirators or rocking beds. However, once the acute illness had passed a patient's ventilatory demands usually decreased rapidly so that emancipation to lesser respiratory aids or to respiratory independence could be started promptly.

Successful convalescent regimens are going to vary in specific methods depending upon the experience and preferences of different staffs. Our own program's paramount aim was to assist the patient in developing maximally all his residual abilities so that he could pursue as far as his powers permitted a normal way of life encumbered as little as possible with mechanical devices of any type. Total emancipation from artificial respiratory aid although emphasized in this program was subordinated to this more comprehensive goal of rehabilitation.

Although all patients were eventually freed from depending upon the tank respirator not all patients were able to dispense completely with respiratory aid after poliomyelitis. Many factors influenced the rate and degree of recovery but the most important were the degree of

damage to the peripheral and central respiratory neurones, the level of general health, the amount of pulmonary and thoracic complications, the level of ventilation both in the tank and in lesser aids and finally the psychological attitudes of the patient. Since these five factors were so important in determining the ultimate therapeutic results they will be discussed in some detail.

*The degree of damage to peripheral and central respiratory neurones.* Since the muscular act of breathing is complex it is usually only crudely analyzed. For clinical purposes the respiratory function of each patient was evaluated in terms of vital capacity, diaphragmatic activity, intercostal activity and whether or not accessory muscles were employed during quiet breathing. These tests have been shown to provide an accurate functional appraisal of ventilatory capacity. No patient became completely emancipated from artificial respiratory aid when he still used accessory muscles to carry on quiet breathing. In general, complete emancipation from artificial respiration during sleep was impossible until over 20 to 30 per cent of the predicted normal vital capacity had returned. Whether a patient with a vital capacity between 25 per cent and 50 per cent became completely free of the need for respiratory aid at night depended on two things: first, the distribution of residual functioning neuromuscular units and second, the status of central respiratory control. Patients whose residual respiratory muscles were largely diaphragmatic breathed with less effort and became free of the respirator at lower vital capacity levels than did patients whose residual breathing was largely intercostal in origin.

Two patients with bulbar poliomyelitis showed evidence of permanent defects involving their central regulation of respiration. The upper two tracings in Figure 370 are the pneumograms of these subjects who had great difficulty in sleeping without artificial respiratory aid despite maximal breathing capacities of over 30 per

cent and 50 per cent of the predicted normal. When they did become sufficiently fatigued to sleep they demonstrated irregularity in respiratory rhythm, abnormal retention of carbon dioxide with  $p\text{ACO}_2$  as we see here ranging on the one hand to 55.4 and on the other hand to 50.7 and choreiform activity of the head and extremities. Additional studies demonstrated low responsiveness to carbon dioxide as a respiratory stimulant and in addition more than 20 per cent reduction in ventilation while breathing 100 per cent oxygen. A larger group of subjects as illustrated in Figure 321 also showed this low responsiveness to  $\text{CO}_2$  as a respiratory stimulant and these are findings which are similar to those observed by Dr Bengtsson in Stockholm 7 years ago. However the very flat curves of the two subjects AW and FL probably represent central damage rather than merely increase in the work of breathing.

The second major factor then influencing the

rate of recovery was the level of general health. Emancipation to lesser aids or to progressively longer periods of independent breathing was seldom pressed unless patients were afebrile and over the critical autonomic lability which accompanied acute poliomyelitis. Gastric tubes and swallowing paralysis did not deter emancipation efforts although trials on rocking beds or in cuirass or positive pressure respirators were delayed until several hours after feeding. Caution was required with a few patients suffering from prolonged swallowing paralysis as they easily developed gastric distention and esophageal regurgitation when fatigued or frightened. Of course aspiration of gastric contents was then a threat.

The third major category was that of pulmonary and thoracic complications. Respiratory infections created insidious and sometimes severe threats during convalescence. Merely a common cold caused the vital capstries of many patients

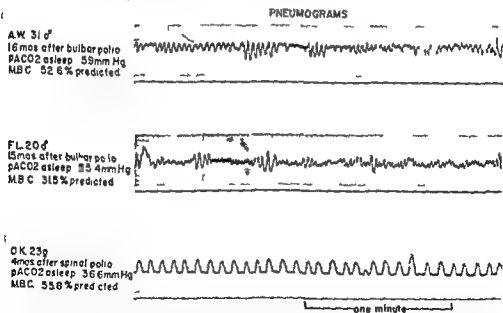


FIG. 320. Chest pneumograms in two patients (AW and FL) with permanent irregularity in breathing following poliomyelitis. Inpiration reads up. The lower tracing demonstrates the chest pneumogram during sleep of a patient who had had spinal poliomyelitis with respiratory failure. The irregularities in respiratory rhythm and abnormally high  $p\text{ACO}_2$  in AW and FL were observed only during sleep when the above tracings were made.

to fall 20 per cent in 30 per cent below their best previous efforts. Tracheobronchitis created more serious problems and two patients with laryngeal paralysis developed obstructing sub laryngeal edema as the result of their ineffective barking coughing activity. Therefore colds bronchitis and other pulmonary infections were treated vigorously in these patients. Those who had mastered glossopharyngeal breathing to the extent of developing a cough proved to have the most effective protection against respiratory infections. When a cold developed respiratory aid had to be increased for almost all patients who were using respirators during any part of the day. Some subjects recently freed from all re

spiratory aid had to be returned to rocking beds or cuirass respirators particularly at night for the duration of the infection. Most patients were placed in postural drainage while rocking. Antibiotics were of less value than steam inhalations postural drainage and inhaled nebulized bronchodilators or wetting agents. Gentle tracheobronchial suction was reinstituted for patients who still had tracheostomies.

Few tracheostomies were closed in these patients if they regained less than 35 per cent of their predicted normal vital capacities. However metal tracheotomy tubes were removed as promptly as possible in convalescence and replaced by the Teflon plugs which we presented

### CO<sub>2</sub> SENSITIVITY FOLLOWING POLIOMYELITIS

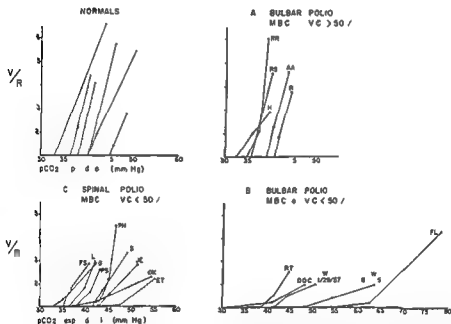


FIG 371 The ratio of evoked minute alveolar ventilation with CO<sub>2</sub> to resting minute alveolar ventilation ( $V/R$ ) with air is given on the ordinate. The  $pACO_2$  is recorded on the abscissa. Subjects breathed room air and mixtures of 3 per cent and 5 per cent CO<sub>2</sub> in air for 20 minutes from a Tissot spirometer. The  $pACO_2$  was measured at the mouthpiece with an infrared gas analyzer. No patient had an MBC or VC of less than 25 per cent. Normals doubled resting ventilation ( $V/R = 2$ ) with a rise in  $pACO_2$  of 15 to 20 mm Hg. Group A doubled ventilation with a mean  $pACO_2$  rise of  $188 \pm 37$  mm Hg. The mean rise in  $pACO_2$  associated with  $V/R = 2$  was  $120 \pm 170$  mm Hg in Group B and  $514 \pm 136$  mm Hg in Group C. Fourteen of the above subjects also were tested for their response to breathing oxygen. Although several showed moderate reduction on oxygen only A W and F L (in B) had more than a 20 per cent reduction in minute alveolar volume on 100 per cent oxygen.

in our exhibit in the other building. These plugs eliminated the irritation of the trachea were cosmetically more attractive and contained an obturator which could be removed immediately to provide access to the trachea.

Pulmonary infections and tracheobronchitis impelled increased caution in respiratory emancipation but did not prevent freeing patients from the tank respirator. Actually in several instances infections could be handled better on the rocking bed than in the tank respirator since rocking with the patient lying prone provided needed pulmonary drainage. Figure 322 shows a young man who had been in the tank respirator for 4 months during which time he had approximately 25 bronchoscopies. These had had the success which may be seen on the left side of the chest. It was possible to get him out of the tank and place him prone on the rocking bed. Therefore it was possible by the simple return to gravitational and postural methods to clear this completely while at the same time moving him from the body respirator.

A significant factor impeding the rate and degree of respiratory recovery was the reduced chest lung compliance suffered by many patients. This increased chest resistance required greater than usual effort or higher respirator pressures to expand the lungs and sometimes accounted for difficulty in obtaining adequate ventilation with certain types of chest respirators. The treatment of this complication has been discussed at length by others; suffice it to say that in our own experience glossopharyngeal breathing was the only effective way of overcoming the problem of increased pulmonary thoracic compliance.

The fourth factor modifying the rate and degree of recovery was the level of ventilation in the tank respirator and in lesser aids. As has been emphasized many patients in tank respirators were comfortable only when hyperventilated. This began during the acute stage of the illness even before the respirator was used and if sustained created a greater ventilatory demand than could be met by chest respirators, rocking beds or other assistive devices. Therefore measurement of tidal volumes and minute volumes in respirators was carried out constantly.

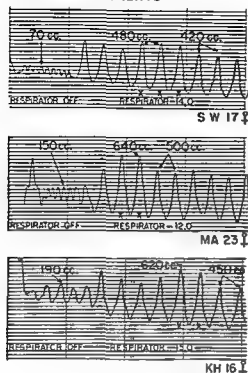
Finally and perhaps in many ways more important the psychological factors modifying



FIG. 377. PA chest roentgenograms on patient R.O.C. The upper roentgenogram (5/27/54) showed changes which reportedly had been present for 3 months. He was placed prone and in postural drainage on the rocking bed and copious amounts of secretions presented at the tracheostomy for removal. (Bottom) By 5/28 the major part of the atelectasis had cleared.

the rate and degree of recovery were considerable. Some patients were much more apprehensive than others over being removed from the respirator. Patients who had suffered periods of anoxia or those whose previous management had been marked by inexperience or uncertainty were particularly frightened by emancipation efforts. It was seldom fruitful to press the apprehensive patient's acceptance of emancipation procedures. Such forced therapy slowed the total recovery process. Engendering self-confidence in the patient sometimes required con-

# ASSISTED VITAL CAPACITY IN RESPIRATOR PATIENTS



X-RESPIRATOR-ASSISTED VITAL CAPACITY

FIG 323 The relationship of assisted vital capacity to unassisted vital capacity in respirator patients. Tracings read from right to left. In each of the three instances illustrated the AVC was measured as described in the text. The respirator was then turned off in these 3 co-operative patients and their unassisted vital capacity was recorded. SW had an AVC of 60 cc, a spontaneous vital capacity of 70 cc. MA had an AVC of 140 cc, a spontaneous VC of 150 cc. KH had an AVC of 170 cc, a spontaneous VC of 190 cc.

considerable time but usually succeeded in restoring to the patient rather than to the staff motivation for recovery.

Evaluation to determine the patient's ability to transfer from tank or positive pressure respirator to lesser aids was performed as soon as the acute illness or severe pulmonary complications had passed. Measurements of breathing in the respirator were made at frequent intervals with a spirometer to detect and correct hyperventilation. Next the extent of residual respiratory function was determined.

A common method of determining the amount of intrinsic breathing ability remaining after acute polio is that of opening a respirator portal and visually examining breathing efforts. This is not quantitative particularly at small respiratory volumes and sometimes frightens the patient. We have found the measurement of what we call the assisted vital capacity to be preferable for estimating quantitatively residual ventilatory ability. The assisted vital capacity is performed without opening the respirator and therefore is particularly useful when dealing with apprehensive or seriously paralyzed patients. The tracings in Figure 323 read from right to left and consist first of resting volume in the respirator followed by a maximal deep breath over the respirator's tidal volume followed in these three co-operative subjects by the measurement of spontaneous unassisted tidal volume or vital capacity. The patient is able to increase over the respirator tidal volume almost as much or exactly as much as he is able to achieve on his own when unassisted by the respirator. This was borne out by consecutive trials on over 15 patients for none of whom was the standard error greater than 15 per cent and in whom the average deviation was approximately 12 per cent. Not until the spontaneous vital capacity rose to over 500 to 600 cc did the assisted vital capacity deviate more than approximately 20 per cent from the actual finding in unassisted patients (Table 163). Patients whose assisted vital capacity was 15 per cent or more of their predicted normal vital capacity were rapidly and easily liberated from tank respirators, whereas patients whose assisted vital capacity at the end of the acute stage of poliomyelitis was 5 per cent or less of the predicted normal (Table 164) usually required more cautious transfer to rocking beds or chest respirators.

To turn these to a specific program the first step in the emancipation from the respirator was accustoming the patient to ventilation by means of a positive pressure respirator or cuirass. This was done with the patient kept on the cart of the tank so that if difficulties arose immediate return to the body respirator could be effected.

Patients who demonstrated an AVC of greater than 10 per cent to 15 per cent of the predicted normal or who could breathe spontaneously from 3 to 5 minutes were transferred directly from

TABLE 163 COMPARISON OF ASSISTED VITAL CAPACITY IN RESPIRATOR PATIENTS TO EARLIEST MEASURABLE UNASSISTED VITAL CAPACITY

NAME	AGE	SEX	RESP TIDAL VOLUME	RESP VITAL CAPACITY	ASSISTED VITAL CAPACITY	UNASSISTED VITAL CAPACITY	% DIFFERENCE
FA	15	F	380	500	170	130	-
KH	16	F	450	670	170	190	-
PS	17	F	470	800	380	440	71
SW	22	F	440	510	0	0	-136
ROC	23	M	480	950	470	540	0
LB	24	F	500	940	440	440	130
HS	26	M	530	930	400	470	0
VB	28	F	460	520	60	0	-48
RR	29	M	640	1840	1700	1440	143
MA	30	F	500	640	140	140	106
RS	43	M	500	760	740	800	0
HA		F	340		470	480	125
Mean						816	

the tank to a hospital or a rocking bed. However as a rule they were first familiarized with positive pressure breathing. It proved easiest to adapt patients to a cuirass or positive pressure respirator first and then work from this to progressively longer periods on the rocking bed.

The choice of which respiratory assistive device to employ for the convalescent patient depended upon his residual respiratory function and on his activity. We preferred to use rocking beds for sleeping and resting. Chest respirators or positive pressure devices were employed for activities such as sitting. Hubbard tank physiotherapy or ambulation. The rocking bed provided most patients with a great sense of per-

sonal freedom. Its motion was comfortable and stimulating and it eliminated the need for much of the repeated moving and positioning which paralyzed patients otherwise required. Most patients learned to rock while lying prone which facilitated pulmonary and renal drainage. As improvement took place the bed was employed for sleeping and other respiratory devices were progressively eliminated during the day.

The two factors which most impeded the elimination of respiratory aid were the number of irreversibly damaged anterior horn cells and the reduction in chest lung compliance. Although we specifically avoided making con-

TABLE 164 RECOVERY OF VENTILATION TRACED BY ASSISTED VITAL CAPACITY

PATIENT	DATE	RESP TIDAL VOLUME	RESP VITAL CAPACITY	ASSISTED VITAL CAPACITY	UNASSISTED VITAL CAPACITY
KH	17/7/53				
16 F	1/1/54	540	630		
Acute	1/8/54	480	600	80	Unobtainable
onset	1/14/54	510	670	170	Unobtainable
17/7/53	1/27/54	530	660	110	Unobtainable
	7/15/54	450	610	130	100 (approx)
		440	800	160	190
MA	7/22/54			360	30
23 F	7/7/54	440	50		
Acute	7/7/54	490	600	80	Unobtainable
onset	7/7/54	500	640	110	Unobtainable
7/5/54	7/30/54	500	700	140	140
	8/9/54	500	0	160	160
		480		40	-40

lescence an endurance contest all patients were maintained on a program of progressively increasing their independent breathing times to tolerance. Other therapeutic methods designed to strengthen the respiratory muscles were extremely disappointing. Occasionally mastery of the technique of effective glossopharyngeal breathing was followed promptly by a rise in vital capacity of 30 to 50 per cent but this probably reflected increased chest compliance rather than improvement in strength of breathing muscles.

It was our practice never to force to exhaustion those patients who claimed they needed assistance to sleep without respiratory aid no matter what the measurement of their vital capacity. Gradually reducing respirator pressures or rocking bed during sleep helped a few patients in overcoming the need for nocturnal aid but it was usually more successful to wake the patient at 4 A.M. turn off the respirator device at that time and urge him to return to sleep. The dark and quiet ward, the recent sleep and the knowledge of constant supervision reduced to a minimum stimuli which otherwise would contribute to arousal.

Although our major emphasis in the paper has been on a program for respiratory emancipation it should be stressed that our goal in the rehabilitation of patients with respiratory insufficiency was more encompassing than the elimination of respirators. Some patients never recover enough breathing function to become completely independent of respiratory aid. Others were marginal and it was found that they were capable of much more active daytime programs if they were assisted at night with artificial respiration.

Many of the patients who are referred to a respirator center are sent from other hospitals many months after illness because they have failed to become free of respiratory aid. In many instances elimination of all use of the respirator has been set as the foremost goal of therapy. Having failed to reach this goal these patients have suffered a deep sense of defeat and a long period of transition is required to redirect them toward potentially achievable objectives. However once they accept the principle that a respirator is no different from any other crutch many of them can make remarkable strides. Seventy patients have been discharged from our

unit in the past 3 years. 18 still require partial respiratory support but none require a tank respirator and most use a rocking bed only while sleeping. Despite their partial dependence upon artificial respiration 12 of these 18 are actively engaged in either schooling, housework or gainful employment. All are home and all are active socially. These are encouraging results and justify the care and effort required to develop a comprehensive program of respiratory emancipation and rehabilitation.

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## DISCUSSION

DR AARSVOLD In weaning the patient from respiratory aid we have mainly followed the principles mentioned by Dr Plum. However, whereas Dr Plum's patients in the acute stage were mostly ventilated in the tank type respirators, approximately one half of our patients were ventilated through tracheostoma by means of different positive pressure ventilators.

This was due partly to the lack of a sufficient number of tank respirators and partly that we have received many of our patients from the provinces, and until 2 years ago we did not have any portable tank respirators. Now we have one but will still probably have to use positive pressure sometimes for transportation because of the difficulties in the communications in Norway.

In contrast to the other Scandinavian countries we in Norway now prefer ventilation by means of tank type respirators when we have the facilities for it.

We have used the pressure ventilators Aga-Bang, Lundia and Engstrom. With each of them we have obtained sufficient ventilation. However, one of the main reasons for our preferring the tank type respirators to the pressure ventilators is that the latter to a greater degree needs trained and observant nurses. In regard to the weaning from the respirator, even those who are most enthusiastic about overpressure ventilators admit that emancipation from this type is much more difficult than weaning from a tank. I have as yet no satisfactory explanation for this. Perhaps the reason is a greater tendency to hyperventilate when we use a positive pressure respirator.

A common experience to anyone working in the field of artificial respiration will be that the longer a patient is ventilated by means of one type of respirator, the more difficult it will be to make him accept another type.

Therefore the first step in weaning patients ventilated by a pressure respirator has been to shift them from one type of respirator to another. Thus they realize that it is possible to breathe in different types of respirators.

On the acute stage has passed and the spon-

taneous ventilation has improved to the extent that the patient can use respiratory aid not giving as effective a ventilation, then we try to use chest respirator and rocking bed. We use the rocking bed as early as possible, hoping to prevent perhaps two of the most common complications in respirator patients: kidney stones and thrombophlebitis. We have also tried to prevent kidney stones by giving enough fluid to keep the specific gravity of the urine below 1015; we have used salicylamide, and we have used the standing bed as early as possible, but nevertheless most of them have got kidney stones. We have discussed the possibility of giving anti-coagulants to prevent thrombophlebitis but have dropped it because of the danger of bleeding if a tracheotomy has to be performed.

We have had the same experience as stated by Dr Plum, namely that psychological factors can greatly influence the weaning of a patient. Some years ago we considered it unthinkable to place several respirator patients in the same room and consequently each had his own. Now we have learned how wrong this attitude was. When the patients are together with other patients at the same stage of recovery, this gives keen competition which can give astonishing speed to the process of weaning. This is one of the reasons for treating patients in a respirator center instead of having them scattered about in the provinces with only a few patients in each hospital.

Dr Plum mentioned that the patients who progressed the furthest were usually those who started in a comprehensive program of respiratory rehabilitation promptly at the end of their acute illness. We have had similar experience but we have learned also that even those who start late may gain important improvement through effective therapy. During the last years patients have been sent to us from the provinces where they have been ventilated by means of positive pressure ventilators for as long as 3 years without any improvement; in the last 2 years. However, when they have been treated together with other patients and have made use of the equipment at the center to full extent

and of the experience of the personnel there we have seen definite improvement after a few months even in cases in which patients have been ill for as long as 3 years. This has caused us to keep the patients at the respirator center for a considerably longer time than before. Formerly we thought that after 1 year we could not obtain any further improvement but now we know that even after several years there can be important improvement through effective therapy.

Another reason for keeping the patients in the center for a long time is that in the beginning for psychological reasons they are less co-operative. Another example of the importance of the psychological factors is the striking improvement we often see when the patient has visited home for the week end. Therefore we try as early as possible to get them home or at least outside the hospital for week ends and later on for longer leaves. In that way we hope to avoid their getting tired of the treatment. They need vacations now and then but according to our experience we do not discharge them finally before several years have passed.

When the patients have learned frog breathing we are much more liberal in regard to sending them home for the week end. We value frog breathing very much and therefore systematically try to teach it to the patients as early as possible. Besides making the patients feel safe in knowing that they can manage without a respirator we regard frog breathing as being important in preventing reduced compliance. Even with patients who have been artificially ventilated for several years we have seen improvement in ventilation capacity after they have learned frog breathing. Glossopharyngeal

breathing can partly substitute for an attachment on the tank for deep respiration. Naturally we are careful not to let the patients exaggerate the frog breathing so as to give circulatory failure.

We also regard frog breathing as being the best method for preventing respiratory complications during the weaning period. If the patient is an effective frog breather we are not as strict as Dr Plum who demands 35 per cent of predicted vital capacity before decannulation. We have removed the cannula on adults with a vital capacity of 300 cc and as yet we have not had to do a retracheotomy. Even if all precautions are taken it is impossible to prevent infection in the respiratory tract now and then but by means of frog breathing tank with vacuum cleaner and postural drainage we have always succeeded in removing the secretion caused by common colds.

We think it is important to remove the tracheal cannula as early as possible. Even when the cannula is made of the least irritating material it will act as a foreign body in the trachea and will cause some secretion. As long as the patient has the cannula there will be consequent secretions.

We also try to avoid as early as possible suction by means of catheters inserted in the trachea because of the traumatizing effect of the catheter. Therefore we teach the patients to get rid of the secretions by means of frog breathing or by tank respirator with vacuum cleaner and postural drainage. We have not considered Teflon plugs as necessary.

Like Dr Plum we do not think that the main thing is to get rid of respiratory aid. We prefer the patients to be able to do work with a respirator instead of lying quietly in bed without respiratory equipment.

# Circulatory Disturbances in Life-Threatening Poliomyelitis

DR WILLIAM A SPENCER

I trust that my remarks will be received strictly as opinions. Disturbances of actions of the heart and of the systemic and pulmonary circulation contribute to death processes in the polio patient with viral invasion of the brain stem or with conditions leading to an asphyctic state such as failure of breathing. The conditions of grave concern in our experience include pulmonary edema, hypotension, vasomotor lability, peripheral circulatory stasis and hyperthermia. Irregularities of cardiac rhythmicity and myocardial insufficiency occur. The complex nature of these conditions suggests that it would be useful first to consider practical experience with diagnosis and treatment of major circulatory abnormalities and then to make a very brief presentation of some of the patterns of disturbances observed in the patient.

The experience to be described has been based upon cardiovascular disturbances observed in a regional respirator center which admits acute patients and has admitted over 1200 since its opening. Without consecutive clinical experience and physiologic investigation one might not be aware of the pitfalls of generalization. We believe that we recognize some of these pitfalls and they are resultants of the very complicated behavior of the cardiovascular system in severe polio. We think this behavior is a consequence of disturbances of the neural regulation of heart action and circulation, biochemical disturbances of the extra and intracellular fluid compartments, or mechanical malfunctions which arise in the course of the disease, its complication or even of its treatment.

Table 165 indicates the frequency of cardiovascular disturbances observed in what we call life-threatening poliomyelitis (after Dr Lassen). In 1000 paralytic patients which we studied 357 were considered to be severely ill, approximately 35 per cent. The most common complication was respiratory muscle paralysis in 72 per cent of the severely ill. Impairment of swallowing of a severe degree occurred in 60 per cent dis-

turbances of respiratory regulation in 27 per cent and approximately 35 per cent had disturbances of circulation. Now this group of 176 individuals who had circulatory disturbances of one or several types accounted for 52 of the 59 fatalities in the entire life-threatened group including those admitted dead on admission for whom it would be impossible to categorize the cause of death. It should be inferred that mor-

TABLE 165 FREQUENCY OF CARDIOVASCULAR DISTURBANCES IN PATIENTS WITH LIFE-THREATENING POLIOMYELITIS

CATEGORY	INCIDENCE PERCENTAGE	
Total severely ill patients	357	
Respiratory muscle paralysis	257	72
Impairment of swallowing	213	60
Disturbances of respiratory regulation	96	27
Disturbances of circulation	126	35

These conditions are mutually exclusive in combination. In the same individual the physiological impairment is indicated by the anatomical location of the disturbance. Nearly all the patients considered to have had either respiratory muscle paralysis or impairment of swallowing or both conditions. The number of patients requiring ventilation is not requiring positive pressure and have been included because of the tendency of allowing impairment of cephalic or major circulatory disturbance. 55 and 14 are included with respiratory paralysis, judgment to be sufficient to require breathing aid but usually difficult in combination with swallowing impairment and vascular problems. In sent signs and symptoms of impending death. The overall series of the group is indicated by the need of respiratory aid for periods exceeding 72 hours in 80 of the individuals. The respiratory muscle paralysis. Tracheotomy was necessary in 10 of the paralyzed patients because of way obstruction or the combination of respiratory muscle paralysis requiring artificial respiration and in 40 of them the swallowing difficulties alone. The table included patients with bulbar poliomyelitis with involvement of the pharyngeal muscles. The patients with involvement of the pharyngeal muscles and in a few instances with lingual paralysis were set apart from the others. It should not appear to be severely ill.

TABLE 166 TYPE AND RELATIVE FREQUENCY OF CIRCULATORY DISTURBANCES OBSERVED IN 357 POLIOMYELITIS PATIENTS AT RISK TO LIFE

TYPE OF DISTURBANCE	PERCENTAGE
Cardio-regulatory disturbances and vasomotor lability	8
Hypertension of any degree	69
Myocardial insufficiency	58
Hypotension	50
Cutaneous vasodilation and hyperthermia greater than 105° F (rectal)	34
Gastro-intestinal bleeding in matings or melena	7
Pulmonary edema	11
Total number of individual conditions or multiple disturbances	176

These results show that the incidence of the individual disturbances is as follows: Hypertension 69%, Myocardial insufficiency 58%, Hypotension 50%, Cutaneous vasodilation and hyperthermia greater than 105° F (rectal) 34%, Gastro-intestinal bleeding in matings or melena 7%, Pulmonary edema 11%. The total number of individual conditions or multiple disturbances is 176.

It appears to go hand in hand with these disturbances of circulatory function. This is simply a statistical association and not proof. However, it does corroborate our clinical relationship in which one sees the occurrence of these conditions at the time of rapid deterioration of the patient.

Table 166 indicates the simple numerical incidence of the various complications mentioned. I did not construct percentage figures because of the extreme variability. The most common condition simply numerically was cardiovascular disturbances and vasomotor lability. Hypertension of any degree, not necessarily severe in the life-threatened group, occurred in only 69 individuals; myocardial insufficiency in 58; hypotension in 50; and so on. Cutaneous vasodilation with hyperthermia up to 105° or higher greater than 105° in 34 individuals; massive gastro-intestinal bleeding in 7; and clinically evident pulmonary edema in 11 individuals.

TABLE 167 TYPE OF DISTURBANCE AND ASSOCIATED MORTALITY IN 126 LIFE-THREATENING PATIENTS WITH CIRCULATORY ABNORMALITIES AND RESPIRATORY MUSCLE PARALYSIS OR SUFFICIENTLY IMPAIRMENT

TYPE OF DISTURBANCE	ASSOCIATED MORTALITY	PERCENTAGE
Pulmonary edema	89	11
Hypertension	2	50
Vasodilation and hyperthermia	68	68
Gastro-intestinal bleeding	48	2
Myocardial insufficiency	36	58
Cardiorespiratory disturbances	35	78
Hypertension	27	69

Total incidence of circulatory disturbances 176  
Total number of individuals 176  
Fatalities 59

From the 126 patients with life-threatening type defined before and not overall expectations with severe paralytic poliomyelitis. In Table 167 these simple numerical incidence figures have been rearranged according to the percentage of associated mortality in order to call attention to those conditions which require most urgent treatment or prevention. Here one sees that the least common condition in our experience is pulmonary edema, of which there were 11 examples and 89 per cent expired.

Next in order of importance with respect to associated mortality was hypotension, then peripheral skin vasoconstriction of severe degree associated with high body temperatures. GI bleeding, myocardial insufficiency, cardiorespiratory disturbances and last, hypertension.

Table 167 simply indicates the percentage of fatal type with a particular complication regardless

of its frequency and should be taken to indicate only the likelihood of associated death and not the complication itself as the cause of death which is quite often the result of many causes.

The development of cardioregulatory and vasomotor disturbances in association with lower brain stem involvement of the swallowing centers has been our general experience both clinically (Table 168) and as a result of neuropathologic examinations of the brain stem. On the other hand the occurrence of circulatory disturbances with respiratory insufficiency in its terminal stages is also a matter of common clinical experience with the signs and symptoms of asphyxia. One sees the same hypotension, the same disturbances of cardiac regula-

tion in both circumstances. Separation of the events then of disturbance of cardiovascular function does not necessarily separate causes from effects or from associated conditions. Certainly functional disturbance of the central nervous system can contribute to these conditions. For example biochemical alterations of hypoxemia, rapid shifts of intercellular and extracellular pH, concentration carbon dioxide—all have been shown to disrupt neuronal function. So once the central nervous system mechanism is disturbed either directly by invasion of the virus or indirectly by virtue of biochemical disturbances, the end results may be quite similar. Also it is true that these latter changes may be compounded with what we

TABLE 168 CARDIOVASCULAR ABNORMALITIES OF MAJOR SIGNIFICANCE IN ACUTE SEVERE POLIOMYELITIS

CONDITION	DIAGNOSTIC CRITERIA	% INCIDENCE*
<b>Regulatory Disturbances</b>		
<b>Heart</b>		
Sinus tachycardia	Peripheral pulse rates above those expected for temperature. Severe tachycardia greater than 200 in children and 180 in adults. Occurs in less than 5%.	27
Sinus bradycardia	Sudden and transitory decrease in pulse rate to 80 beats per minute or less with fever. Often associated with increasing temperature of the body and hyperthermia.	17
Wandering auricular pacemaker	Changing PR interval in the ECG, usually transitory or associated with conduction defects. Not grave unless there is hypoxemia.	7
Premature beats of ventricular origin	Premature complexes in a series may be of grave significance if there is diminished pulse volume or dropped beats.	6
Supraventricular tachycardia	Paroxysmal rapid rates or may remain rapid and unmodifiable.	1
<b>Systemic circulation</b>		
Hyperthermia	Temperature elevations in excess of 105 F° (rectal) — 40.5 C. Often coincides with severe generalized cutaneous vasoconstriction.	24
Cutaneous vasoconstriction	Patchy, purplish mottling of the skin with cutaneous cyanosis and sluggish circulation. Skin temperature is cold. Occurs just in the hands and feet with hyperthermia in children. Grave if generalized.	22

\* Incidence figures are relative and are based on the last 100 poliomyelitis patients admitted to the respiratory center with a threat to life due to respiratory muscle paralysis or swallowing difficulty. Mortality was 9% in this group of patients. 55 patients had swallowing difficulty, 29 of these were combined with respiratory muscle paralysis; the remainder had respiratory muscle paralysis alone. 73 patients required prolonged respiratory aid.

# Circulatory Disturbances in Life-Threatening Poliomyelitis

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TABLE 168 CARDIOVASCULAR ABNORMALITIES OF MAJOR SIGNIFICANCE IN ACUTE SEVERE POLIOMYELITIS (Continued)

CONDITION	DIAGNOSTIC CRITERIA	PERCENTAGE INCIDENCE
Systolic and diastolic hypotension	Systolic pressures less than 90 mm in the febrile adult and 60 mm in the child. Often associated with moribund hypoxemia, terminal asphyxia or artificial respiration in the presence of vasomotor incompetence.	15
Systolic and diastolic hypertension	Systolic pressure greater than 200 mm of mercury in adult and 160 mm of mercury in children. Lesser degrees are self-limited unless indicating progress or elevation. Severe degrees can precede pulmonary edema and congestive failure.	10
Pulmonary circulation	A condition of increased bronchovascular markings in the lungs during artificial respiration. P <sub>1</sub> as a right axis P vector rotation and clockwise rotation of the QRS vector.	12
Pulmonary congestion	Acute respiratory distress with most crepitant rales in the lungs. Bronchiolar constriction may be present especially if hypoxemia develops. Pink frothy secretions fill the trachea following severe hypertension.	7
Pulmonary edema	Poor soft quality of the heart sounds. Rapid heart rate or gallop rhythm may be present. Cardiac enlargement may not be observed. ECG indicates separation of the QRS and T waves. ST segment depression. Prolongation of QT time. T waves are shape changes and low voltage. First indicated by serial changes in successive ECGs. ECG and heart sound registration show marked shifts in the duration of mechanical systole (onset QRS to beginning of second heart sound). Can be secondary to pulmonary complications: hypoxemia, electrolyte alterations and myocarditis.	17
Myocardial Disturbances	Often occurs with ileus and gastric atony. May follow episodes of severe bradycardia. Blood tinged or chocolate gastric secretions. Melena.	20
Myocardial insufficiency		
Autonomic Disturbances		
Gastrointestinal bleeding		

would like to call regulatory or compensatory adjustments to the actual disturbances taking place in the circulatory system. For example if the blood pressure falls this calls into play immediate reflexes from the carotid sinus mechanism which tend to elevate blood pressure toward its normal value. When this circumstance occurs at a time when the central nervous system apparatus is damaged you can see how complicated the interpretation or the results may be. Clinical investigation and experience may tend to substantiate one or another cause of circulatory disturbances as prepotent in any individual patient or at a particular time. It is probable that processes of the disease physiologic effects of the treatment procedures and

the aggregation of complications have a variable timetable.

Figure 374 shows one clinical example which reveals the chaotic disturbance of circulation with which you are all quite familiar. This was a 28-year-old man who had total muscular paralysis swallowing impairment and required artificial respiration. In the upper half of the chart the solid black dots connected by a continuous line are a record of the systolic blood pressure which proceeded from high levels of 190-200 mm of mercury and over a course of some hours gradually declined to low levels and then spontaneously recovered until terminally when the blood pressure suddenly dropped and the patient expired in a matter of 8 minutes.





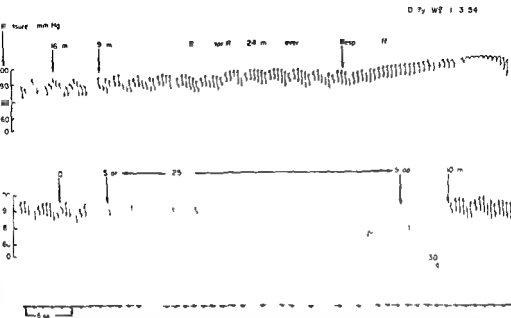


FIG 375 The genesis of bradycardia, hypotension and autoregulatory disturbances by hypoxemia in bulbospinal poliomyelitis. Percentage saturation of femoral arterial blood is indicated on the right of the figure and pulse rate values on the left. Representative ECG's A, B and C coincide with 40, 60 and 80 minute time intervals. Equivalent ventilation volumes in both tank and cuirass respirators were assured by spirometry. The patient was confined in the tank respirator except for the period indicated, cuirass. Blood pressure values are indicated along the abscissa.

stant artificial respiration due to respiratory muscle paralysis and severe swallowing difficulty. This individual  $\square$  be sure had evidence of severe brain stem invasion with his swallowing difficulty but had a fairly stable heart and blood pressure picture until the time that we introduced for a brief period a cuirass respirator for nursing care. In this chart on the vertical dimension the pulse rate is indicated by the hollow circle connected by broken lines. The arterial oxygen saturation of the femoral blood by direct sampling, not by oximeter is indicated by the solid black circle connected with an uninterrupted line. Above are represented electrocardiograms in the boxes marked A, B and C. Blood pressure  $\square$  indicated here in the control period at the time of hypoxemia and following hypoxemia. The patient was changed from the tank respirator where he had essentially normal arterial oxygen saturation and a pulse rate of 120 to 130 and then into a cuirass

respirator for a period of about 8 minutes. Arterial oxygen saturation fell to 60 per cent although the level of ventilation remained the same. Associated with this was a decline of pulse rate from 130 to approximately 80 beats per minute. The ECG indicated the manner of cardiac slowing and the ECG components following the hypoxemic episode did not return to the previous configuration. After prompt return to the respirator and the temporary use of high oxygen concentration his former status was not quite resumed. Now we have a patient who has undoubted evidence of brain stem involvement but who was reasonably stable until accidentally subjected to hypoxemia and it unveils disturbances of regulation of these vital functions that we have just seen.

Figure 376  $\square$  a continuous femoral arterial pulse recording from a 7 year-old child who had swallowing difficulty. She had sufficient right diaphragm and intercostal paralysis requiring

artificial respiration for a period of 4 weeks. On the surface I noticed that this patient had marked lability of skin color varied from blanching to suffusion and that the blood pressure seemed to vary obviously with the cycling of the respirator which of course is not unfamiliar to many in this audience. On the vertical dimension of the chart the calibration of the blood pressure is in mm of mercury. The tank respirator cycling was at minus 16 cm. Pay attention only to mean blood pressure because we do not feel this recording is accurate enough to interpret pulse pressure. Mean blood pressure tends to be around 90 minus 16. It goes higher when the respirator pressure is decreased to minus 9 and then when the respirator is turned off the blood pressure goes on off the record although it tends to come down immediately. In the lower record we increase the negative

respirator pressure to minus 25 and you see a decrease in mean blood pressure which is quite significant in approximately 30 seconds. Now the point here was that this patient required careful minimal respirator pressures at higher rates to minimize the circulatory effect of pressure breathing probably coming about because of vasomotor incompetence and inability of the person in this circumstance of polio to adjust venous return to the thorax in the condition of pressure breathing. Now briefly the conditions in order of importance that one would diagnose are pulmonary congestion which is a condition of increase bronchovascular markings in the roentgenogram decreased distensibility of the lungs ECG changes Pulmonary edema by our clinical definition is occurrence of pink frothy secretion in the tracheobronchial airway with acute respiratory distress. The next most im-

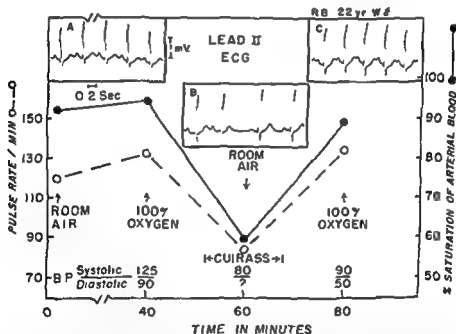


FIG. 326 The effect of tank respirator pressure breathing on the femoral arterial blood pressure in a poliomyelitis patient with vasomotor incompetence. Sections of directly recorded femoral arterial blood pressure are indicated in relation to tank respirator pressures and the cessation of artificial respiration. The blood pressure values are shown in relation to pressure calibration in mm of mercury on the ordinate at the left of the scale. Time calibrations are along the abscissa of the record and apply except where gaps are indicated. Cyclic variations are due in part to the placement of the pressure transducer outside of the chamber of the respirator. Pulse pressure fluctuations are to be considered only representative and not true values because of the small caliber of the arterial needle.

portant condition we mentioned was that of hypotension which we recognize as systolic pressures in the adult of less than 90 mm of mercury in the state of fever and 60 mm of mercury less than 80 in the child. Cutaneous vasoconstriction is self-evident. However it is misleading. We believe that it in fact contributes to decrease heat loss from the skin and that is why it is so commonly associated with elevation of body temperature. The regulatory disturbances are self-evident. Myocardial disturbances I would like to discuss briefly. Disturbances of cardiac activity in the course of polio are being recognized with increased frequency. This undoubtedly reflects electrodiagnosis in appreciation of lesser degrees of myocardial insufficiency rather than frank congestive heart failure. The most useful measures for us have been serial pulse and blood pressure records of the type appreciated by the anesthesiologist. Careful serial electrocardiograms and simultaneous phonocardiograms for measurement estimation of systolic duration routine fluoroscopy and occasionally special studies of the type shown in Table 169 are recommended.

TABLE 169 INCIDENCE OF CHANGES IN SERIAL ELECTROCARDIOGRAPHIC AND PHONOCARDIOGRAPHIC MEASUREMENTS IN ACUTE POLIOMYELITIS WITH THREAT TO LIFE

MEASUREMENT AND CONDITION	% INCIDENCE
<b>SCALAR</b>	
PR interval prolongation	6
QRS interval prolongation due to bundle branch block	9
QT interval prolongation	28
QT interval shortening	7
T wave flattening	5
U wave deflection	17
<b>VECTOR (Direction and magnitude)</b>	
Right atrial preponderance	15
Left atrial preponderance	6
Right ventricular preponderance	22
Left ventricular preponderance	3
<b>SYSTOLIC DURATION (Relative to heart rate)</b>	
Systolic prolongation	11
Systolic shortening	41

These figures are based on the study of serial electrocardiogram and phonocardiogram in 100 acute poliomyelitis patients with threat to life.

Now the ECG is important to us because in a careful study of 350 individuals shown in Table 169 approximately one fourth show QT interval prolongation 17 per cent U wave deflection about one fourth right ventricular preponderance in the vector analysis and 15 per cent right atrial preponderance. Nearly half of the individuals showed marked shortening of systolic duration corrected for the rate of the heart. In about 10 per cent systolic prolongation occurred.

In the present state of our knowledge it is obviously difficult to correlate these different changes with pathologically demonstrable lesions in the myocardia or conduction system. However that the changes have an extreme chronologic variation in the same individual suggests the possibility that they are related to current dynamic changes of strength of contraction ischemia or state of nutrition of the muscle metabolic disturbances of the cells and indeed even altered neural regulations.

Satisfactory treatment can be successfully carried out in some individuals (Table 170). Survival in the presence of usually lethal complications must be attributed to many factors and the relationship of favorable results to specific treatment viewed with considerable caution. However the declining incidence of circulatory catastrophe may be cited. It has paralleled earlier use of artificial respiration better management of airway problems improvements in respiratory equipment and its usage employment of physiologic measurements to estimate ventilation the avoidance of overtreatment. Furthermore I think the prompt correction of hypotension with such drugs as Levophed administered with great care the use with proper indication of digitalis derivatives the use of Rauwolfia serpentina extracts at appropriate times careful fluid and electrolyte management the avoidance of overloading of the circulation the prompt correction of pulmonary complications and many other things put together appear to have some benefit in these dreadfully complicated circumstances. I do not have time to go into detail in our particular plan of treatment but I would be delighted to see any one of you at our exhibit and discuss with you the management of these conditions which as I have inferred are quite complicated. I would like to say in conclusion that

TABLE 170 TREATMENT OF CARDIOVASCULAR ABNORMALITIES IN ACUTE POLIOMYELITIS

CONDITION	TREATMENT	DOSAGE
Sinus tachycardia	Usually no therapy used unless extreme (See text)	
Sinus bradycardia Autonomic disturbances such as gastro intestinal bleeding	If persistent use vagal blocking agents Methantheline bromide Oral medication indicated through nasogastric tube if gastro intestinal bleeding is present Also use constant nasogastric suction Atropine is contraindicated Transfusion for blood loss	Banthine® 5-10 mg administered parenterally Oral dose 25-50 mg every 6 hours for 24 to 72 hours Amount depends upon hemoglobin and hematocrit
Disturbances of cardiac rhythm	Usually no treatment unless hypoxemia is the cause Oxygen therapy with adequate pulmonary ventilation	
Hypertension	Indicated if systolic pressure exceeds 180 mm of mercury in the adult and 150 mm of mercury in the child Extracts of Rauwolfia serpentina for parental use Hydralazine and hexamethonium derivatives contraindicated	Rescinnamine® 160 Micro grams/kg of body weight /74 hours One fourth calculated amount given immediately in intravenously balance in divided doses every 6-8 hours
Hypotension	Drug therapy indicated if systolic pressure falls to 80 mm of mercury Norepinephrine Other vasopressors less effective or contraindicated	Levophed® 4-8 mg per 1000 ml of intravenous solutions Rate of administration depends upon pressor response
Myocardial insufficiency	Pressure adjustments of the pressure breathing device in respiratory patients See text for important general considerations and indications for drug therapy  Digitalis derivatives Administer cautiously hypersensitivity to effect may be present  Rauwolfia serpentina derivatives	See text for technique of adjustment of respirators  Dioxotin® 0.25 to 0.10 mg total dose/kg of body weight in one 24 hour period Administer one fourth immediately Control succeeding amounts by electrocardiographic evaluation (See text) Balance in 6-8 hour intervals in divided doses  Rescinnamine® Parenteral dosage same as treatment of hypertension First parenteral dose may be followed by oral administration of 0.25 mg of Serpasil® every 6 hours
Hypothermia	Treatment indicated for body temperature in excess of 104° F (rectal) alternate application of warm and cool sheets to the body with brushing of the skin to stimulate circulation Usually needed if hypertension is coexistent  Alcohol may be used intravenously Acetylsalicylic acid not indicated Avoid alcohol rubs and ice packs unless hypothermia is desired	Alcohol 5% solutions in 5% and 10% dextrose or invert sugar in water 500-1000 ml may be given intravenously depending upon age

TABLE 170 TREATMENT OF CARDIOVASCULAR ABNORMALITIES IN ACUTE POLIOMYELITIS (Continued)

CONDITION	TREATMENT	DOSEAGE
Acute pulmonary edema	Establish adequate pulmonary ventilation with intermittent positive pressure breathing apparatus and administer high concentration of oxygen. Drug therapy may be helpful. Rotation of tourniquets on the extremities. If treatment is unsatisfactory, nebulization of aerosols may be tried. Electrophrenic respiration if available. Treat myocardial failure if present.	Aminophylline 1 to 5 Gm administered slowly intravenously with 5 to 50 ml of 50% glucose

See also Effect of treatment of the infection and dosage of the sedative in determining duration of treatment.

the pathologic physiology occurring in poliomyelitis complicated by respiratory insufficiency or swallowing difficulty is variable. Careful and constant individualization in the planning and execution of remedial preventive measures is of extreme importance. The timing and use of such measures or indeed the withholding of treatment are clearly matters of judgment, experience and investigation of abnormal functions.

It is hoped that these "experiments of nature

in pathologic physiology will no longer result from the invasion of the nervous system by the poliomyelitis virus. The advent of a successful vaccine widely employed clearly ensures this eventuality. It is hoped then that descriptions of the chaos brought by this infection can then be relegated to obscurity except for those principles that are axiomatic and apply to any catastrophic illness posing a threat to life through respiratory and circulatory dysfunction.

# Biochemical Changes in Poliomyelitis Patients

DR INGEMAR JUNGNER

The first large scale biochemical investigations which serve to establish the premises for rational care of poliomyelitis patients were made by Bower *et al*<sup>1,5</sup> in connection with the wide spread epidemic in Los Angeles in 1948. Their comprehensive studies disclosed severe biochemical changes particularly with respect to the acid base and electrolyte balance and nitrogen metabolism. The experience of Bower *et al* was subsequently put to good use in the large epidemics which broke out in Scandinavia—in Copenhagen in 1952<sup>6</sup> and in Stockholm in 1953.<sup>7,10</sup> This also applied to the considerable methodologic and technical improvements introduced by Astrup *et al*<sup>11,12</sup> for the laboratory control of patients with disturbances in the acid base balance.

As far as the acid base balance is concerned the usual disturbances are due to the altered gas exchange. The acidosis and alkalosis which then arise are thus *respiratorily* conditioned and are to be ascribed primarily to alterations in the CO tension (pCO<sub>2</sub>) of the blood. The organism has only a limited defense against these changes in the degree of acidity. Therefore it attempts to maintain a constant pH in the blood—i.e. to normalize the ratio of bicarbonate to CO<sub>2</sub> in Henderson Hasselbalch's equation—by a compensatory adaptation of the blood content of bicarbonate. Consequently exact determinations of the CO<sub>2</sub> tension are a prerequisite for therapeutic

measures in cases of respiratory insufficiency. This presupposes adequate laboratory resources irrespective of whether the tension is determined directly or is calculated indirectly from exact determinations of the pH and bicarbonate content.

In the usual conditions of metabolic acidosis or alkalosis the plasma bicarbonate is on the contrary the first to undergo a change compensatory normalization of the pH being brought about by a change in the CO<sub>2</sub> tension. Consequently with a metabolic disturbance alone it is often possible in practice to rely only on determinations of the plasma bicarbonate measured as total CO<sub>2</sub> actual bicarbonate CO<sub>2</sub> combining power or standard bicarbonate (Table 171).

The conditions become still more complicated if both respiratory and metabolic disturbances are present.<sup>14,16</sup> This is not uncommon in poliomyelitis although the incidence seems to vary appreciably in different epidemics. For example in the 1952 epidemic in Copenhagen a relatively large number of patients had a marked rise in nonprotein nitrogen and an element of renal acidosis.<sup>6</sup> In the Stockholm epidemic in the following year we had on the contrary a very low incidence of such complications<sup>7</sup> and the changes were marked in only a few cases (Fig. 327). However the incidence of metabolic acidosis has been considerable in later sporadic

TABLE 171 PLASMA BICARBONATE

SYMBOL	UNIT	DEFINITION
Total CO <sub>2</sub>	mMol/l	Total content of CO <sub>2</sub> in blood or plasma estimated after expulsion through addition of acid
Actual bicarbonate	mMol/l	Bicarbonate content of plasma at actual pCO <sub>2</sub>
CO <sub>2</sub> Combining power	mMol/l	Bicarbonate content of plasma separated at the actual pCO <sub>2</sub> of blood and then equilibrated at pCO <sub>2</sub> = 40 mm Hg
Standard bicarbonate	mMol/l	Bicarbonate content of plasma separated from fully oxygenated blood at 38° C and pO <sub>2</sub> = 40 mm Hg

cases of poliomyelitis. Therefore the possibility of a metabolic element in poliomyelitis should be borne in mind and the chemical analysis aimed at finding out whether or not a metabolic disturbance is present and if so its degree.

The difficulties of evaluating the acid-base balance in cases of poliomyelitis with respiratory paralysis are associated with the fact that the compensatory mechanism of the body cause different conditions to present great similarities. Moreover the relation between pH, CO<sub>2</sub> tension and plasma bicarbonate is a logarithmic one. A feature of practical importance from the therapeutic point of view is that the respiratory shifts in CO<sub>2</sub> tension take place far more rapidly than the compensatory change in the bicarbonate content of the blood. Therefore the conditions are simpler in the acute stage of poliomyelitis and can be clearly presented in the form suggested by Astrup<sup>1</sup>. He utilized the fact that the logarithm of the CO<sub>2</sub> tension shows a linear relation to the blood pH at a certain bicarbonate content. This implies that the primary respiratory changes are independent of the absolute bicarbonate content of a given plasma sample. The metabolic changes in acid-base balance as mentioned primarily cause changes in this bicarbonate content. This offers a possibility to distinguish between the respiratory and metabolic disturbances when they occur simultaneously. For if the bicarbonate is determined under standardized conditions with respect to temperature, CO<sub>2</sub> tension and also the oxygen saturation the secondary respiratory changes are eliminated. A measure of the metabolic com-

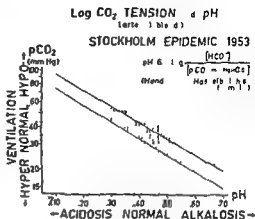


FIG. 377. The appearance of the values obtained in the Stockholm epidemic when the logarithm of the CO<sub>2</sub> tension is given as a function of the pH. As mentioned the logarithm of the CO<sub>2</sub> tension shows a linear relation to the blood pH and it can be inferred from this figure that the region for normal respiratory changes is relatively small and that there is a wide scattering of the values. On the whole they nevertheless fall within the region where purely respiratory disturbances in the acid-base balance are found or in its immediate vicinity. Definitely divergent values, i.e. cases with metabolic imbalance, are few and some of them have a normal pH as an indication of compensation.

ponent alone is then obtained. We have used Astrup's standard bicarbonate, defined as the concentration of bicarbonate ion in the plasma

TABLE 1/2. THE CONTENT OF TOTAL-CO<sub>2</sub>, ACTUAL BICARBONATE, CO<sub>2</sub> COMBINING POWER AND STANDARD BICARBONATE IN PLASMA FROM THE SAME BLOOD SAMPLE AT VARYING CO<sub>2</sub> TENSION AND OXYGEN SATURATION. EXPRESSED AS  $\mu\text{MOL/L}^{11}$

ANALYSIS	PCO <sub>2</sub> 20 MM Hg	PCO <sub>2</sub> 40 MM Hg	PCO <sub>2</sub> 80 MM Hg	SAMPLE
Total CO <sub>2</sub>	16.8	27.2	30.0	Fully oxygenated blood
Actual bicarbonate	16.2	27.0	27.6	
CO <sub>2</sub> Combining power	18.0	27.2	27.5	
Standard bicarbonate	21.0	27.0	27.0	
Total CO <sub>2</sub>	19.6	25.7	34.8	Fully reduced blood
Actual bicarbonate	19.0	24.5	32.4	
CO <sub>2</sub> Combining power	21.0	25.7	31.9	
Standard bicarbonate	27.0	27.0	27.0	



part of fully oxygenated blood at 38° C when the CO<sub>2</sub> tension is 40 mm Hg as a gauge in this respect

As seen in Table 172 the standard bicarbonate is constant at different CO<sub>2</sub> tensions in opposition to the total CO<sub>2</sub> the actual bicarbonate and the CO<sub>2</sub> combining power. Moreover it is seen that according to definition the standard bicarbonate is equal to the actual bicarbonate content at a pCO<sub>2</sub> of 40 mm Hg. The values are taken from a recent work of Astrup where he points out that the oxygen saturation affects the plasma bicarbonate. That is why the plasma has to be separated at a fixed oxygen saturation and suitably separated from fully oxygenated blood.

We have found Astrup's standard bicarbonate to be a reliable and probably the most convenient measure of the nonrespiratory components of the acid base metabolism in the organism.

In Table 173 the different forms of disturbances in acid base balance are summarized. The respiratory acidosis and alkalosis are recognized by changes in the CO<sub>2</sub> tension while the metabolic acidosis and alkalosis affects the standard bicarbonate. The degree of metabolic imbalance is obtained as the difference between the standard bicarbonate calculated and its normal value and is shown in the last column. This difference is negative in metabolic acidosis and positive in metabolic alkalosis.

The metabolic component is of course especially important if the polio cases are complicated by for example diabetes. However I would like to emphasize that an irrelevant factor also can influence these analyses which should be decisive for treatment with artificial respiration. Table 173 shows a case which was not complicated by any organic disease but in which a mental depression brought about hunger acidosis reflected as moderate but fully evident metabolic acidosis.

The necessary data for determining the patient's acid base balance on a given occasion thus are the pH, the CO<sub>2</sub> tension and the standard bicarbonate. Several methods can be used for the analysis. However Astrup<sup>1</sup> has suggested a simple method for facilitating the analysis. This consists of first determining the actual pH of the blood followed by a new pH determination at 38° C and a fixed CO<sub>2</sub> tension suitably 40 mm Hg, either on separated plasma or on fully oxygenated blood.<sup>13</sup> Both the actual CO<sub>2</sub> tension and the standard bicarbonate can then be obtained from diagrams. We have used this method of Astrup's as well as determinations of the blood pH and total CO<sub>2</sub> and found the figures to be in good agreement.

Disturbances in the electrolyte balance in poliomyelitis are due partly to the changes resulting from the altered gas exchange and partly to the

TABLE 173. EXAMPLES OF DIFFERENT FORMS OF DISTURBANCES IN ACID-BASE BALANCE

TYPE OF DISTURBANCE	pH	TOTAL CO <sub>2</sub> mMOL/L	pCO <sub>2</sub> mm Hg	STANDARD BICARBONATE mMOL/L	<sup>a</sup> HCO <sub>3</sub> <sup>-</sup> mMOL/L
Normal values (arterial blood)	7.35-7.45	20-29	35-43	21.7	±2.8 (28)
Respiratory acidosis	7.06	39.5	133	22.9	+1.2
Respiratory alkalosis	7.64	15.7	15	21.6	-0.1
Metabolic acidosis	7.10	5.8	III	6.9	-14.8
Metabolic alkalosis	7.57	28.5	43	36.3	+14.6
Polio patient with occasional hunger acidosis					
Feb. 2	7.36	20.8	37	20.2	-1.5
Feb. 3 A.M.	7.27	21.8	47	19.3	-2.4
Feb. 3 P.M.	7.18	16.8	44	14.9	-6.8
Feb. 4	7.21	19.6	48	17.0	-4.7
Feb. 8	7.37	25.3	44	23.4	+1.7

<sup>a</sup> Difference in standard bicarbonate = standard bicarbonate - 21.

losses through for instance secretions and sweating. Finally they are a result of the raised urinary excretion of potassium and phosphate in particular associated with the excessive destruction of muscle.

Of the changes resulting from impaired ventilation those in respiratory acidosis are the most important. They are associated with an increased urinary output of chlorides as a result of the increase in plasma bicarbonate. However the changes in the chloride content of the plasma are generally so small that subnormal normal values are found. Even if these respiratory conditioned changes are not so marked it is important to take therapeutic measures for their compensation. This is because they are greatly accentuated by considerable chloride loss through such paths as profuse sweating, large quantities of secretion from the respiratory tract and gastric atony.

The urinary excretion of chlorides is not accompanied by any sodium loss but by some retention in the body probably due to passage of sodium into the intracellular fluid. Thus the organism as a whole does not suffer from sodium deficiency but the low values found in both blood and urine are largely to be ascribed to a pathologic distribution of sodium in the body. Consequently an attempt to produce normalization by compensatory therapy may lead instead to an accentuation of this pathologic distribution. Laragh<sup>27</sup> has shown that oral administration of potassium chloride to patients both with and without edema—although not with poliomyelitis—produces a rise in the blood content of sodium. This seems to be of interest in poliomyelitis as well since it is hoped by the administration of potassium to exchange this substance for the increased intracellular content of sodium *ie* to produce the normalization implied by adequate therapy. However the exact distribution of potassium and sodium between the intra and extracellular fluid has not as yet been sufficiently elucidated to permit any definite conclusions.

It is usual in poliomyelitis to find—apart from a passing initial rise—definite fall in serum potassium and a rise in its urinary excretion.<sup>4, 11</sup> The muscle destruction releases large quantities of potassium but there is nevertheless a fall in its serum concentration. Potassium deficiency is generally regarded as being of such

importance that extra potassium is administered routinely. Despite this it often may be difficult to normalize the blood values completely. As Schriber *et al* have stressed it is necessary in the dosage of potassium to recall that through its influence on sodium and thereby on the chloride and bicarbonate content of the blood it will have an acidotic effect. Therefore it will increase any respiratory acidosis and even cause symptoms of intoxication. Consequently the potassium dosage must be individualized on the basis of the results of the chemical analysis taking into account such matters as the state of ventilation, the extent of paralysis and the risk of renal complications.

True overwhelming evidence exists in favor of the increased urinary output of  $K$  as the factor responsible for the fall in serum  $K$ . But the possibility cannot be disregarded of a retention of  $K$  in the muscles as in familial periodic paralysis<sup>28</sup> for example. However in the latter condition the potassium is usually protein bound to a considerably greater degree than normally and is not ultradialyzable. Our analyses have not disclosed any change in the binding of serum potassium in poliomyelitis patients.<sup>4</sup> Nor have ECG studies indicated any marked degree of potassium retention in the heart muscle at any rate.

In poliomyelitis the electrolyte disturbances are somewhat difficult to compensate since the therapeutic range is narrow. The risk of both under and overdosage is great and individualized therapy based on repeated chemical analysis is essential. Experience indicates that the urinary excretion of chlorides should be kept on a fairly low level—below 60 mEq—/24 hours—and that  $Na$  should be administered restrictively and only together with  $K$ . With respect to potassium we have usually given  $KCl$  in doses of 40–80 mEq—/24 hours. Despite this dose or even a higher one no less than 37 per cent of the respirator cases in the Stockholm epidemic still had hypokalemia.<sup>9</sup>

Fluid must be given to compensate for the often very large loss. The risk of edema must not be underestimated since autopsy has disclosed (in addition to myocarditic and other changes) subclinical signs of pulmonary edema<sup>29, 30</sup> even in cases where the analyses have indicated an adequate fluid balance. When large quantities of fluid must be given in the

acute stage it is essential for blood plasma or hypertonic serum albumin solution to be given concurrently by the parenteral route.<sup>3,10</sup> Administration of protein hydrolysates has been recommended<sup>4</sup> but administration of individual amino acids seems to be unfavorable judging by animal experiments.<sup>7</sup>

In lengthy immobilization it is important even in the later stages to maintain a high diuresis. In poliomyelitis as in many other immobilizing illnesses e.g. skeletal tuberculosis the urinary output of calcium is raised.<sup>5b,67</sup> However the blood values are usually normal owing to mobilization of Ca from the skeleton. The phosphate excretion is also raised and the high incidence of urinary concretions in the late stages of poliomyelitis is presumably due to the solubility product of Ca and phosphate being exceeded.<sup>8</sup> The favorable effect of forced diuresis in such cases as in nephrocalcinosis is largely to be ascribed to the fact that it has been maintained throughout the 24 hours.<sup>20</sup> A reduction in the urinary excretion of Ca, P and N is also reported with steroid therapy.<sup>9c,31</sup>

Poliomyelitis is associated with a great disturbance in *nitrogen metabolism*. Owing to fever and inanition in connection with the onset and particularly the excessive muscle destruction due to paralysis the nitrogen balance is markedly negative. A striking feature is that muscle destruction has a far more rapid onset and is more severe in poliomyelitis than in paralysis resulting from division of a nerve or a disease of the central nervous system for instance. The greatly increased urinary excretion of nitrogen may amount to a total 25.45 g/24 hours.<sup>4,9</sup> The maximum excretion is noted 5-16 days after the onset of paralysis. The increase in total nitrogen excretion is represented chiefly by an increase in urea nitrogen whereas creatine nitrogen and creatinine nitrogen play lesser roles quantitatively as do uric acid nitrogen and amino acid nitrogen. The amino acids have been studied but neither their quantity nor distribution has proved to be characteristic in poliomyelitis.<sup>8</sup>

The fact that urea nitrogen is chiefly responsible for the increased urinary output of nitrogen indicates that the breakdown of muscle protein largely follows the normal pattern and is practically complete. Such rapid and complete

breakdown must place great stress on the liver which is forced to deal with a synthesis of urea for instance which greatly exceeds the normal function. Liver damage in poliomyelitis has been reported by several authors<sup>8</sup> even in the form of decreased serum cholinesterase activity with no other clinical signs of liver damage.<sup>32</sup> In this connection it may be mentioned that we have determined the activities of glutamic oxalacetic transaminase, malic dehydrogenase and lactic acid dehydrogenase in serum without finding any marked elevation.<sup>6</sup>

*Creatinuria* is a constant feature in all severe cases of poliomyelitis but is also normally seen in connection with immobilization.<sup>34</sup> In our experience the creatinuria in poliomyelitis has a surprisingly lengthy duration—several years—in relation to the relatively short period of very high urea excretion. Several authors have found a relation between creatine excretion and muscle reduction but Hoagland, Gilder and Shank<sup>35</sup> have pointed out that the decreased output of creatinine is a better measure of the reduction of muscle mass in progressive muscular dystrophy. In our experience this also applies to poliomyelitis. Under normal conditions the creatinine excretion is strikingly constant as compared with the large variations shown by other urinary components under the influence of such factors as fasting, consumption of meat and muscular exercise. The creatinine coefficient i.e. the excretion in mg/kg of body weight per 24 hours is given as about 23 under normal conditions. We have found this coefficient to be decreased in poliomyelitis in one of our cases it was as low as 7 mg/kg/24 hours. A coefficient in relation to the muscle mass alone—and verified for poliomyelitis patients—would be of great value as an objective gauge of the extent of paralysis. Also it would be of interest to analyze the enzymes in the muscles regulating the metabolism and function of creatine. In poliomyelitis<sup>4</sup> as in muscular dystrophy<sup>36</sup> there is a disturbance in creatine metabolism so that a large proportion of an oral dose of 1 to 3 grams is recovered in the urine whereas normally the whole amount is utilized.

Provided that renal function is unimpaired the values for the blood content of urea and creatinine show little variation. To find out the way in which the muscle breakdown products

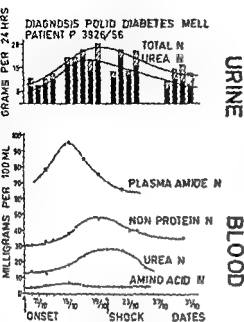


FIG 378 Case of poliomyelitis with diabetes mellitus

reach the liver Astrup *et al*<sup>1</sup> analyzed the amino acid content of the blood but found no rise. We have analyzed the various nitrogen fractions in the blood of poliomyelitis patients but were also unable to find any change in the amino acid content. Nor did we detect any signs of degraded muscle protein or sufficient quantities of unaltered myoglobin to explain the considerable nitrogen transport. On the other hand the amide nitrogen fraction of protein exhibited such a large rise during the acute stage of severe poliomyelitis that it could account for the nitrogen transport.<sup>2</sup>

Figure 378 shows a case of poliomyelitis with diabetes mellitus. In the acute stage there is a considerable increase of total nitrogen and urea excretion in the urine. Of different nitrogen fractions investigated in the blood the plasma amide nitrogen shows an increase in the early stage. Later in connection with a diabetic coma and shock there was also an increase of the nonprotein nitrogen and the urea nitrogen. The amino acid nitrogen is within normal limits throughout the muscle destruction associated with poliomyelitis.

The negative nitrogen balance is reflected as

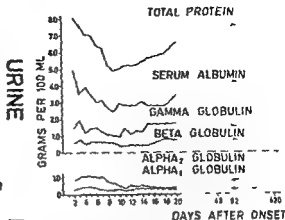


FIG 379 Serum proteins in a case of severe poliomyelitis (paper electrophoresis Veronal buffer pH 8.6)

1. Decrease in serum albumin<sup>1</sup> The decrease is in proportion to the nitrogen deficit and thus to muscle destruction and is a constant finding in severe cases. The total serum protein decreases but the greatest variation occurs in the albumin fraction. This starts to fall soon after the onset and reaches a minimum during the 5th to the 10th day. In severely paralyzed cases it even falls below the edema limit (about 3 g/100 ml). We have made paper electrophoretic analysis in a large number of cases. We then found no significant changes in the other serum fractions apart from a moderate rise in the gamma globulin fraction 2 or 3 weeks after the onset and an irregular increase of the alpha globulin (Fig 329).

The decrease in serum albumin has been presumed to depend on impairment of liver function but it should probably be seen also against the background of very rapid metabolism. Like liver protein serum albumin has an average half-life of only 10 days as compared with about 80 days for other body protein. When the organism is exposed to increased nitrogen breakdown as in high fever, widespread necrosis, burns and obviously poliomyelitis as well, it reacts by a decrease in protein synthesis with a resulting protein deficit. This is expressed as a decrease in protein in the whole organism although it is most conspicuous in the serum albumin fraction. According to calculations made by Elman<sup>2</sup> a decrease in serum albumin

of 1 Gm represents a loss of 30 Gm of protein for the body as a whole. Also it must be recalled that such a protein deficit particularly if it is of long duration is invariably accompanied by such vitamin and mineral deficiencies that they must be provided by supplementary nutrition.<sup>43</sup>

Although the poliomyelitis virus has long been regarded to have a particular affinity to nervous tissue, relatively few data are available on the biochemical changes in the central nervous system in this disease. The possibilities of such investigations are obviously limited. Moreover the experience gained through analysis of the cerebrospinal fluid is not of such a nature that it does not as yet give any conception of the biochemical changes in the individual nerve cells. Nevertheless some observations have been made which may be of diagnostic value, e.g. with respect to the pyruvic acid content<sup>39, 40</sup> phosphatase activity<sup>41</sup> and inorganic P content.<sup>4</sup> In addition certain changes in the cerebrospinal fluid have been found in the form of a raised protein content<sup>42</sup> and increased nuclease activity<sup>44</sup> which can be ascribed to some destruction of nervous tissue.

Unquestionably there are many other biochemical problems of great interest in poliomyelitis. For example it is possible that sialic acid (neuraminic acid) plays an important role for the affinity of poliomyelitis virus to the central nervous system, as has been shown for the influenza virus.<sup>4, 46</sup> Studies of sialic acid-containing mucoproteins inhibiting virus reception in host cells would then be of interest in view of recent discussions regarding the spreading of the poliomyelitis virus in the body.

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Dr Lewis Dr Spencer's comprehensive report of cardiovascular phenomena associated with severe paralysis is of major medical significance. One important feature is the demonstration again of the complexity of the poliomyelitis lesion in patients who can now be salvaged. His data give a true profile of the incidence and variety of neurologic and myocardial lesions causing circulatory disturbance in severely stricken patients. Fortunately the development of satisfactory methods of care has preserved lives and the parallel growth of diagnostic techniques has made it possible to delineate pathologic processes in the ward and clinic rather than in the pathology laboratory.

Dr Spencer's paper leaves little room for comment except commendation and an expression of appreciation for the information which he has abstracted from so large a series of cases and made available. As a passing comment it seems noteworthy that the patients with extreme cardiac and cardiorespiratory abnormalities studied by Dr Spencer's group were frequently admitted to the respiratory center many hours after the onset of life threatening disease. Therefore it is difficult as he pointed out to determine what part of the pathologic process was due solely to the invasion of the poliomyelitis virus and what to the effect of secondary phenomena such as hypoxia. In our own work with patients we have been impressed with the decline in frequency of fulminating disease and extreme disability although by no means its disappearance among those whose care we have been able to supervise from the first stages of illness. There seems to have been progressive increase of salvage potential and diminution of total paralysis among patients who have received co-ordinated medical care from the start. Patients who are transferred to centers only when their lives are endangered by the direct or secondary consequences of poliomyelitis almost invariably already have experienced a significant deterioration of prognosis.

Just as poliomyelitis can produce a wide variety of anatomic lesions and functional disturbances so can it also cause biochemical altera-

tions of great diversity among individuals and seemingly different phenomena among affected populations as well. When the reports of biochemical investigations from Los Angeles, Winnipeg, Copenhagen and Stockholm are compared there are many similarities but some important differences in the recorded data. In considering these data it is important to bear in mind that the disease under investigation may be different not only because of varying virus virulence and immunity status of the population but also as a result of a multiplicity of other factors. These include the availability of treatment facilities, the distances over which patients must be transported and particularly the nature of diagnostic and therapeutic techniques which are applied.

With the rapid decline in the incidence of poliomyelitis perhaps many of the problems which have been raised in the past will not be solved. However great contributions already have been made.

Dr Jungner has pointed out forcefully that both respiratory and metabolic influences affect the acid base balance. A question was raised as to whether or not there was a metabolic facet of poliomyelitis which is determined by the character of the virus in different epidemics, for example in Copenhagen as opposed to Stockholm. To answer this question it would be necessary to have accurate appraisals of many factors in both epidemics. It would be interesting to have comparative data on carbohydrate administration during the critical phase of illness in such a comparative group of patients. The higher frequency of renal acidosis and elevated nonprotein nitrogen in the earlier Copenhagen epidemic might have been accountable on the basis of marked caloric deficit with increased protein catabolism and possible ketonemia. It may be significant that marked renal acidosis and elevation of nonprotein nitrogen in the blood is not found frequently even among the most severely involved patients under the relatively easy circumstances which prevail in advanced treatment centers in the United States at least during nonepidemic years. Almost certainly such complications would occur if facili-



ties and personnel were put to the extreme test of managing outbreaks comparable to those which occurred in the Scandinavian countries in recent years

To maintain the patient with respiratory paralysis at relative homeostasis the principal objectives are well known to be normal arterial oxygen and carbon dioxide tensions and normal pH. The methods of achieving this vary from country to country. We have had no experience with Dr Astrup's method but similar information is of course obtainable by chemical analysis of the blood by determination of the arterial blood pH by use of the Rahn Fenn diagram for the determination of arterial  $\text{CO}_2$  and by various other methods which are all based upon the Henderson Hasselbalch equation

With respect to electrolyte balance the complexities of electrolyte phenomena are adequately indicated by Dr Jungner's discussion. It is impossible to comment on every detail of the problem which he has so well explored but specific items will be selected for further consideration

The hypokalemia which Dr Jungner attributed principally to increased urinary output of potassium may be influenced by another factor in our opinion. Hyperventilation alkalosis has been shown to result in depression of serum potassium. The mechanism is probably the shift of potassium ions into cells and the resulting depression of serum potassium level contributes to that which is due to potassium loss in the urine and other fluids such as saliva

Dr Jungner did not stress problems relating to sodium. In our experience low sodium syndromes have constituted more difficult metabolic problems than hypokalemia. Also this seems to have been the experience in Winnipeg as reported by Thomson. He found that low serum sodium values were often encountered in patients with or without evidence of respiratory failure. It was discovered also that reaction by high sodium intake led to excretion and in some instances to restoration of sodium level in the serum accomplished with moderate sodium intake. The mechanisms involved here are not clear but may include a true metabolic shift in these patients resulting from a receptor mechanism.

Another and more likely mechanism is hyponatremia due to depletion of intracellular potassium with or without resultant change in extracellular potassium level. This intracellular hypokalemia may lead to low serum sodium levels either by intracellular sodium transfer or more likely intracellular to extracellular water shift. In either instance total body sodium is normal. Under these circumstances administration of additional sodium leads to an increase of extracellular fluid which may prove disastrous. The hypotheses stated presuppose a depletion of intracellular potassium i.e. a loss greater than that which would be accounted for merely by the destruction of muscle. The potassium to nitrogen ratio in the urine must be greater than the potassium to nitrogen ratio of muscle tissue

Dr Jungner referred briefly to the relationship between calcium and phosphorus metabolism and the formation of calculi in the urinary tract which has been investigated by Dr James Elliot and our other associates in our laboratory. The magnitude of the clinical problem is well known in the extent of the metabolic derangement as indicated by the observations of Plum and Dunning and demonstrated prolonged calcium loss even in relatively mild bulbar paralysis

By means of petrographic analysis with the polarized light microscope it has been possible to identify the mineral content of calculi occurring in recumbent patients. Struvite and apatite are the commonest constituents occurring in poliomyelitis. Almost all of the renal and bladder stones are composed of these two minerals that is basic or tertiary calcium phosphate and magnesium ammonium phosphate. Approximately 5 per cent are calcium oxalate. An interesting fact concerning the formation of stones is the relationship between solubility of calcium phosphate and pH of the urine. In simple aqueous solution calcium phosphate precipitates at pH 6.2 or higher. Apatite precipitates at pH 6.6 or higher. Brushite is the most soluble compound and is found rarely in kidney stones. You begin to find apatite when the blood calcium levels are low. Struvite is of importance in the history of the disease. It causes urinary calculi and may impair renal function.

tance Above pH 7.2 struvite crystallizes from urine which is saturated or supersaturated with calcium and phosphate. These findings indicate that in addition to hypocalcemia an abnormally high urinary pH must be maintained for apatite or struvite calculi to develop. The mean pH of normal 24 hours urine is 6.1.

Our work has demonstrated that the commonly held view that urine is a supersaturated solution of calcium phosphate is not true. In most normal persons the urine is undersaturated with calcium phosphate and this is true also of many patients with severe paralysis due to poliomyelitis. However normal urine may be supersaturated as evidenced by the finding of brushite and apatite crystals in normal urines. Likewise the urine of paralyzed patients may be and often is saturated or supersaturated with calcium phosphate. Factors which favor the precipitation of calcium salts for the formation of calculi are the high pH mentioned above, the presence of foreign bodies such as catheters and the presence of infection. In some instances infection with urea splitting organisms is the basis for the elevated pH but this is by no means always the case. As a matter of fact the mechanism for persistent alkaline pH's is not well understood.

Protein metabolism is a subject which warrants a great deal of discussion and further investigation. Our own studies have been concerned principally with determination of creatinine clearances and creatinine phosphate ratios. In late convalescent poliomyelitis we found a consistent reduction of serum creatinine with levels of 0.7-0.6 mg/100 ml as compared to normal values of 1.0-1.5 mg/100 ml in controls. Endogenous creatinine clearance also has been found to be impaired. Whether this is due to a renal lesion or a defect in creatinine metabolism remains to be determined.

The empirical observation of a significant fall in serum albumin level during the initial few weeks of illness is interesting. We have not investigated this problem. However the interpretation of the fall of serum albumin level is extremely difficult. Without an estimation of albumin pool size and a knowledge of average half life no deductions concerning albumin turnover are possible. In one patient in late convalescence studied in our laboratory by use of labeled serum albumin there was no demon-

strable abnormality of albumin turnover. Attention should be called to the fact that various methods of labeling albumin lead to different estimations of half life and pool size. The 10 day half life mentioned by Dr. Jungner is now considered by almost all workers in the field to be short by 5 to 15 days.

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Dr. Saxe: I shall discuss one topic of the remarkable report of Dr. Jungner, namely the control of artificial respiration.

Using a blood pH electrode that permits the determination of the blood pH with very small quantities of blood we have studied the relationship of capillary and arterial blood and the interdependence of blood pH and total  $CO_2$  of serum in purely respiratory cases, metabolic cases and cases with respiratory and metabolic disturbances. The blood pH measurement is extremely delicate because of the little changes that

might be significant. The determination must be correct to 1/100 of a pH unit and contact with air must be strictly avoided during the whole procedure. Also all anticoagulants including oxalate and heparin must be avoided because of their acidifying effect upon the blood pH which is enhanced in hypoproteinemia. In addition the temperature of the blood should not undergo any change at any time. We regulate the temperature of the electrode to exactly the patient's temperature within 1/10° C and measure the blood pH within a few seconds as a routine bedside determination. Blood (5 to 10 microliters) is drawn into the thermostabilized and carefully shielded capillary electrode which is regenerated after each measurement by drawing pepsin solution into it. The regeneration is accomplished in 2 minutes. Before and after the measurement the electrode is checked with at least 2 certified buffer solutions of which the values are known to 1/100 of a pH unit at different temperatures.

The total CO content is measured by the microgasometer of Natelson permitting a determination in 30 microliters of serum.

In 20 patients capillary blood closely approached arterial blood with respect to pH and CO when a good hyperemia of the ear lobe or fingertip is ascertained. The first drop is always discarded.

All normal subjects had pH values of 7.38 to 7.42 whereas the venous blood shows pH values lying 0.02 to 0.03 pH units lower than the corresponding arterial or capillary blood values.

In purely respiratory acidosis CO accumulates and the pH values fall very rapidly. The critical values according to our experiences are pH 7.20 to 7.22. At this moment artificial respiration must be installed if the pH falls below this limit irreparable damage of the respiratory centers occurs. On the other hand metabolic acidosis may show much lower blood pH and values of 7.07 to 7.10 have been observed in uremia which seemed well tolerated. In these cases the total CO is far below normal. Therefore it is extremely important to differentiate between respiratory and metabolic disturbances both of which may occur in poliomyelitis. For this reason the total CO must be determined and the CO tension calculated according to Henderson and Hasselbalch.

Practice has shown that in purely respiratory cases mainly in the acute phase of poliomyelitis blood pH values are a sufficient guide for the equilibration of the patient especially as they can be repeated easily very often. The logarithm of the CO tension shows a linear relationship to the blood pH.

It is of extremely high importance to reach respiratory equilibrium as soon as possible i.e. in the very first hours of respiratory disturbance and to maintain very carefully this equilibrium. The virus infection we believe reaches higher centers in inadequate respiratory conditions than when the patient is perfectly equilibrated and of course oxygen given in a sufficient amount. A slight hyperventilation is preferable to an even small hypoventilation where traces of CO are accumulated with every breath.

# Pulmonary Complications in Poliomyelitis

DR HANS NORDENSTAM

Patho-anatomically the pulmonary complications in poliomyelitis present only a few types in a natural way they can be divided into 3 groups

1 Complications following respiratory paralysis and/or airway obstruction. The conditions constituting this group seem to be causally connected and are aspiration, emphysema, atelectasis and petechiae from suffocation.

Many authors state that widespread atelectasis develops frequently in respiratory poliomyelitis in spite of adequate respiratory treatment and that it is the main cause of death in a number of cases. The results of the controlled respiration not quite as successful as expected have been explained *inter alia* by paralysis of the ciliary movements in the mucous membranes of the bronchi out of reach of the suction tube and of the usual bronchial cleansing. In addition

the secretion is assumed to be displaced still more toward the periphery through the variations of the respiratory pressure and finally fill out the alveoli thus interfering with the exchange of the gases. In the present material genuine atelectasis could be diagnosed patho-anatomically in only 8 cases.

Table 1/4 shows all permanent complications and the cases are ranked according to clinical symptoms.

2 The second group consists of mechanical injuries and inflammatory changes.

I must talk about one type of inflammatory change namely interstitial pneumonia. In the monograph of 1956 we had low microscopic incidence of bronchopneumonia. Microscopically we found about three times as much as macroscopically. This is one of the reasons for a new investigation which was made this year and

TABLE 1/4 INCIDENCE RATES OF PULMONARY COMPLICATIONS RELATED TO OTHER ORGAN SYSTEMS AND TO DIFFERENT CLINICAL GROUPS

INCIDENCE RATES OF PULMONARY COMPLICATIONS RELATED TO  
OTHER ORGAN SYSTEMS AND TO DIFFERENT CLINICAL GROUPS

RESPIRATORY SYSTEM

REMAINING ORGAN SYSTEMS

Clinical groups	Atelectasis	Emphysema	Aspiration	Suffocation hemorrhages	Decubital ulcers in hypopharynx and trachea	Tracheobronchitis	Bronchopneumonia of conventional type	Interstitial pneumonia	Extravasation of erythrocytes into the alveoli	Macrophages containing hemodermin like pigment	Pulmonary edema	Hydrothorax	General and relative dilatation of right ventricle of heart	Myocarditis	Pulmonary embolism	Erosions of gastric mucosa acid malacia in esophagus/stomach
I	1	2	0	5	3	4	1	10	10	10	10	10	10	10	1	3
Ia	2	1	2	1	2	3	1	2	3	2	2	2	3	3	0	3
II	3	6	1	5	4	8	5	7	7	6	8	4	8	8	2	5
III	2	5	3	4	0	4	0	4	5	4	3	0	2	5	0	3
Totals	8	14	6	15	9	19	7	23	25	22	23	16	23	26	3	14

543

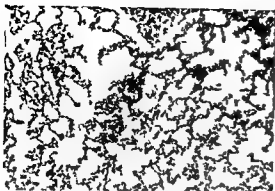


FIG 330 Hydropneumonia and capillary hyperemia (van Gieson's stain) ( $\times 20$ )

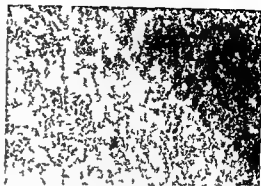


FIG 332 Interstitial pneumonia and hydropneumonia. Large amounts of erythrocytes in the alveoli (van Gieson's stain) ( $\times 20$ )

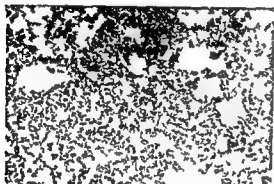


FIG 331 Interstitial inflammatory infiltration of the lung. Marked capillary hyperemia and a few erythrocytes in the alveoli (hematoxylin-eosin stain) ( $\times 20$ )

now inflammatory changes could be demonstrated in 73 cases out of 26 fatal cases of poliomyelitis. Also we could differentiate between 2 types of inflammatory alteration namely patchy bronchopneumonia of conventional type and interstitial changes simulating virus pneumonia. Microscopically we had 7 bronchopneumonia cases which showed the ordinary varying appearance the alveoli engorged with fibrinous exudate and inflammatory cells above all polynuclears.

The interstitial or pneumonia type was observed in 23 cases. Only microscopic study will disclose it and mild degrees will be concealed easily by more conspicuous phenomena namely excessive erythrocytic engorgement of the alveoli, extreme dilatation of the capillaries in the alveolar walls, emphysema or atelectasis. The interstitial infiltrations are often closely interwoven with intra alveolar fibrinous exudate and abundant polynuclear leukocytes.

Histologically the interstitial infiltrations resembling those of several types of virus pneumonia are mainly composed of lymphocytes and monocytes with very few polynuclears. The infiltrations show an arrangement in clusters and streaks around vessels and nerves partly following fine bronchial branches which appear encased in the same way by lymphocytes and monocytes. However the infiltration ceases in the peripheral parts of the bronchial wall and the remaining layers present no signs of inflammation unless a coexisting purulent bronchitis is demonstrable. The vessels of the alveolar walls show excessive dilatation often giving an erroneous impression of general thickening of the walls due to other causes. Large numbers of erythrocytes intra alveolar as well as interstitial and abundant macrophages containing pigment similar to hemosiderin occur frequently presumably owing to the extreme dilatation of the vessels and extravasation of red cells. Figures 330 and 331 show interstitial pneumonia with heavy pulmonary edema dilatation of capillaries in the intra alveolar walls. Figure 332 also is an interstitial pneumonia or pneumonia with a great many red blood cells intermingled in the alveoli.

3 The third group are called hydrodynamic disorders in the peripheral respiratory tract. Patho-anatomically this group is an entity consisting of pulmonary edema only as mentioned. However it is an important group quantitatively and yields interesting information concerning the character and pathogenesis of poliomyelitis. Not until recent years has the hydrodynamic disorder in itself been considered an important

complication. Simultaneously the attitude toward them is undergoing a change as present they are looked upon by many as a result of the poliomyelitis directly equivalent to the paralysis of striated muscles rather than a secondary complication. However this interpretation of the pulmonary edema is to a great extent a revival of the former concept of damage to cerebral autonomic centers.

Before the era of antibiotics and controlled respiration pneumonia and atelectasis were the most important pulmonary complications as to incidence (as well as cause of death). After the introduction of modern antibiotic treatment atelectasis was said to constitute an overwhelming group and as regards fatalities to rank foremost beyond comparison. Great expectations were aroused by the improvement of the respiratory technique and of the management of controlled respiration. However pulmonary edema was reported as early as in the 1930's but practically only in connection with pneumonia atelectasis encephalitis and after 1941 myocarditis. There are quite remarkable differences as to incidence rates in series of otherwise similar cases of fatal poliomyelitis. A brief account will be given of three theories on the pathogenesis of pulmonary edema in poliomyelitis. These are (1) paralysis of cerebral autonomic centers (2) atelectasis (3) the cardiovascular pathogenesis.

In a previous paper I have called the pulmonary edema in poliomyelitis hydropneumonia because of its differences from ordinary pulmonary edema. It is rather difficult to explain this high degree of edema but the demonstration of chromaffin granules in characteristically shaped cells in human skins and other tissues makes possible a different explanation not discussed previously. Next to the skin pulmonary tissue has hitherto showed the greatest proportion of these cells. Also in vessels arteries and veins as well as capillaries they occur rather abundantly. In human skin the cells show long protrusions containing the chromaffin granules. We have found that these granules can go away when the skin is irritated in some way and we have made injections of different things such as histamine and the histamine releaser P 48. Then we can see how the granules go away for some minutes.

The experiences described provide the basis for a different pathogenetic concept of pulmo-

nary edema and hydropneumonia in poliomyelitis. It may arise from capillary leakage caused by essentially peripheral neuromuscular paralysis due to the interstitial pneumonitis throwing the transmitters out of function with subsequent blockage of constricting impulses to the vessels. The chromaffin cells having neural character embryologically could reasonably be invaded by the poliomyelitis virus in accordance with its neurotropism or predilection for structures deriving from the neuroderm.

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# Gastro-Intestinal Complications in Poliomyelitis

DR HAROLD N NEU

Although the reports in the literature of the effect of the poliomyelitis virus on the neuro muscular system are voluminous few concern gastro intestinal complications. In fact in the *Bibliography of Infantile Paralysis* from 1789 1944 with over 8 000 references collected there are no reports of complicating gastro intestinal ulcerations. The recent reports have largely been concerned with the complications seen in the acute bulbar poliomyelitis.

Saphir has emphasized that there is no gastro intestinal lesion characteristic of poliomyelitis. The gastro intestinal lesion which occurs in acute bulbar poliomyelitis probably results from damage to the hypothalamus. The association between brain damage and changes in the stomach was of course first noted by Kammerer in 1821 and later by Rokitsansky in 1841. The experimental investigators have as early as 1867 and since confirmed that damage to the anterior portion of the hypothalamus involving the tubular region results in gastric hemorrhage and erosion. Furthermore its destruction results in the impairment of parasympathetic activity with

altered digestion inhibited peristalsis and marked distention.

Dr Baker of the University of Minnesota amplified the existing knowledge by detailed study of 115 autopsied cases of bulbar poliomyelitis from which the complete hypothalamus was available. 16 of these patients had manifested gastric bleeding during their acute illness and at the same time Dr Baker and his group noted that 61 per cent of the patients with bulbar poliomyelitis had some destruction of the hypothalamic nuclei.

All observers agree that in the patients with acute poliomyelitis presence of these gastro intestinal complications must be regarded as a grave omen seriously affecting the prognosis for the patient.

In this study we have been more interested in the gastro intestinal complications of poliomyelitis patients respiratory and postrespirator in the convalescent and chronic phases. In order to evaluate the extent of these complications the applications for admission to the respirator and rehabilitation centers of the United States during

TABLE 175 GASTRO INTESTINAL FUNCTION OF ALL APPLICANTS FOR ADMISSION TO REGIONAL RESPIRATORY AND REHABILITATION CENTERS FEBRUARY 1955 TO FEBRUARY 1957\*

COMPLICATIONS	TOTAL ALL APPLICANTS	PATIENT CAN SWALLOW			
		NOTHING	LIQUIDS	SEMI SOLIDS	SOLIDS
Total all applicants	529	29	19	59	477
None	441	2	7	47	335
Gastric tube	39	22	8	6	3
G I bleeding	6		1		5
Gastric dilatation	22			3	19
Gastric tube and G I bleeding	3	3			
Gastric tube and gastric dilatation	5	1	2		2
G I bleeding and gastric dilatation	12	1		3	8
All three	1		1		

\*Based on applications for admission to Regional Respiratory Center (NFIPT Form 64) received during the period February 1955 to February 1957.

TABLE 176 GASTRO-INTESTINAL FUNCTION OF ALL APPLICANTS FOR ADMISSION TO REGIONAL RESPIRATORY AND REHABILITATION CENTERS FEBRUARY 1955 TO FEBRUARY 1957  
DIVIDED INTO THOSE UNDER 15 YEARS AND THOSE OVER 15 YEARS\*

COMPLICATIONS	TOTAL, ALL APPLICANTS	PATIENT CAN SWALLOW			
		NOTHING	LIQUIDS	SEMI-SOLIDS	SOLIDS
Total Applicants Under Age 15	91	8	2	12	69
None	76	1		10	65
Gastric tube	7	5	2	2	
G I bleeding					
Gastric dilatation	3				3
Gastric tube and G I bleeding	1	1			
Gastric tube and gastric dilatation	1	1			
G I bleeding and gastric dilatation	1				1
All three					
Total Applicants Aged 15 and Over	438	21	17	47	353
None	365	1	7	37	320
Gastric tube	30	17	6	4	3
G I bleeding	6		1		5
Gastric dilatation	19			3	16
Gastric tube and G I bleeding	2	2			
Gastric tube and gastric dilatation	4		2		2
G I bleeding and gastric dilatation	11	1		3	7
All three	1		1		

\* Based on applications for admission to Regional Respiratory Center (NFIP Form 64) received during the period February 1955 to February 1957.

the 25 month period of February 1955 to February 1957 were reviewed (Table 175). There were over 529 applications which included a small number (less than 5%) of multiple applications which were received from patients who had applied to more than one center. The analysis is of course complicated but those applications which did not specify the gastro-intestinal status of the patient were excluded from the tabulation. In summary 50 per cent of the patients had some type of gastro intestinal complication from the onset of their illness. Only 11 per cent of them had the gastric tube at the time of application. Further in Table 176 when these are broken down between the ages of 15 and under and those who are 15 and over approximately the same proportions (84% and 83%) reported neither gastric tube, gastro-intestinal bleeding nor gastric dilatation. Since most of these patients were already in the chronic and convalescent phase before applica-

tion to these centers these statistics clearly indicate that in the chronic poliomyelitis patient gastro-intestinal complications are not as frequent as they are seen in acute poliomyelitis.

In our own Respiratory and Rehabilitation Center we studied 141 respirator or postrespirator patients (Table 177). These patients were chiefly bulbospinal or high spinal types of poliomyelitis. All were in the convalescent or chronic

TABLE 177 GASTRO-INTESTINAL COMPLICATIONS IN 141 RESPIRATOR AND POSTRESPIRATOR PATIENTS ADMITTED TO A RESPIRATORY CENTER

Total No. Admissions	141	100%
Peptic ulcer	2	1.4%
Hemorrhage	5	3.5%
Acute gastric dilatation	8	5.6%
Distention and stasis	24	17.0%

\* Twelfth sewer the first case





FIG 333 Severe dilatation

phase with over 80 per cent in the chronic phase. The onset of their acute illnesses had been from 1 month to 6 years prior to admission to the Center. In age they varied from 6 years to 49 years although the majority were 15-30 years of age.

In the respirator group of patients the vital capacities ranged from 0 to 900 cc. In the postrespirator group they were over 1000 cc but usually under one third of normal predicted vital capacity.

Interestingly only 3 patients had an indwelling gastric tube on admission to the Respiratory Center. The remaining patients could swallow liquids, semi-solids or solids although actually the residual paralysis of the palate and pharyngeal muscles in many patients made mealtime a slow and laborious effort both for the patient and the attendant. Of course since the respiratory problems were paramount an appropriate program was instituted to wean the patients to lesser respiratory aids as soon as feasible and of course it is somewhat in retrospect that we can appraise the gastrointestinal complications which arose.

It may be noted in this table that the 2 cases

of peptic ulcer represent an incidence less than that which occurs in the normal population of this age group. All of these patients were required to make adjustments to situations of catastrophic stress yet in only these 2 were we able to confirm the existence of ulcer. The management in these two cases was uneventful.

Massive gastric hemorrhage with hematemesis was not encountered. However in 5 cases there was sufficient bleeding to produce melena which was confirmed by guaiac tests. 2 of the patients were mentioned previously. Routine stool studies for occult blood were not performed. However since all of these patients were bedfast and over 80 of them were quadriplegics in addition to the respiratory involvement daily reporting of the gross characteristics of the stools was routine.

In our series of patients 8 had acute gastric dilatations of an emergency nature requiring immediate use of a gastric tube with suctioning. In all patients the onset was unnoticed until the symptoms became acute with definite impairment of respiratory function. In 3 of the cases there had been food indiscretion such as the following:

A 26-year-old white housewife who had been maintaining her respiratory status at home with a cuirass respirator and rocking bed despite her vital capacity of 500 cc. one evening ate some hamburger with her family. The other members developed nausea and vomited. The patient however was unable to vomit. She experienced abdominal distention which was so severe it interfered with the successful operation of the cuirass respirator. She showed progressive respiratory embarrassment and was admitted to the Center. On admission severe gastric dilatation was observed. A Levine tube was passed left in place for 24 hours. Intravenous fluids administered. 36 hours later she returned to her home. Figure 333 illustrates the severe dilatation and this has been observed many times in other patients.

Fortunately the awareness of this complication in the respirator patient results in early treatment when the beneficial effects are most apparent. In none of our cases did the problems of severe electrolyte disturbance arise. The elevation of the diaphragm by the dilated stomach impedes respiration to such a degree that acute

respiratory failure may supervene. Dr Baker has felt that many of the delayed deaths of bulbar poliomyelitis may be due to this acute dilatation. Certainly we have suspected that in a number of unexplained sudden deaths of chronic respirator patients in home care situations this may have been a factor.

The most common gastro-intestinal problems in the chronic respirator patient are related to simple stasis and distention. This is evidenced by the frequency with which constipation is noted in a Respiratory Center by the difficulty in securing satisfactory scout films of the abdomen for survey of renal calculi. Dehydration, the low phosphorus acid ash diet may contribute to the problem but more important is the absence of effective abdominal muscle contraction with increase in the intra abdominal pressure.

The immobility did not appear to be significant since impactions were noted more frequently in those patients who were up in wheel chairs as compared with the quadriplegics confined to the full body respirator or to the rocking bed. In 70 per cent of our patients this was sufficiently important a problem to merit active attention of the staff.

It is difficult to determine whether these complications represent residual effects of damage to the hypothalamus or were side-effects of the difficult psychological adjustments required in facing a catastrophic illness. Drugs such as Prostigmin or Pitressin did not have their usual effects in increasing peristalsis in these patients.

When fecal impactions do occur it is important not to exhaust the patient in removing the impacted stool. The use of oil retention enemas has not been any more successful than the use of fecal softener (such as dehydroxyanthroquinone or dioctyl sodium sulfosuccinate) in preventing impaction. In none of our patients had mechanical obstruction requiring surgery occurred.

In conclusion we feel that our experience and study emphasizes that the gastro intestinal complications usually do not have the bad prognosis in chronic respirator patients that such complications imply in the acute phase of poliomyelitis. One exception is acute gastric dilatation which is definitely more frequent in the respirator pa-

tient than in other chronic disabling illnesses. It is impossible on the basis of our experience to postulate that this complication is a residual of injury to the hypothalamus with resultant altered parasympathetic balance yet the residuals of bulbar involvement exist in many of these patients. This observation is contrary to the frequently accepted concept that recovery from bulbar poliomyelitis is usually complete.

Careful day-by-day observations are equally important to the chronic respirator patient in order that gastro-intestinal complications do not further impair the already diminished respiratory reserve.

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DR NELBIRCH In addition to the question of gastro-intestinal complications, our experience permits some further information on the incidence of peptic ulcers although they are encountered only rarely in the acute stage of the disease. Among the 115 patients with poliomyelitis who died in 1952 in the Blegdamshospital, Copenhagen, autopsy revealed peptic ulcers in 8 cases (6 men and 2 women) in these 8 cases death occurred within periods ranging from 3 to 27 days after the onset of the disease. Melena and hematemesis had only been recognized in 1 of the cases whereas the remaining 7 peptic ulcers represented casual autopsy findings; the complication had *not* manifested itself in gastro-intestinal hemorrhage. Stool samples were not examined chemically for blood by routine during the acute stage of the disease. Severe lesions of the brain stem, particularly of the hypothalamus, were demonstrated in all 8 cases.

A particular interest is attached to 5 patients (out of a total of 349) with respiratory insufficiency in whom massive gastro-intestinal hemorrhage was present, i.e. 1 case of hematemesis and 4 of melena; the peptic ulcer just dealt with was found at autopsy of 1 of these patients. Autopsy findings from another of the patients showed no intestinal abnormalities. The 3 survivors, 2 of whom were infants, did not present any signs of gastro-intestinal pathologic conditions. In this connection attention should be called to findings (Bennike and Grandjean) demonstrating that manifest hemorrhagic diathesis may represent a not infrequent complication in patients severely stricken by poliomyelitis, particularly if combined with respiratory paralysis (15, out of a total of 349 cases). Most likely this increased bleeding tendency may be ascribed to a reduced capillary resistance because this was the only demonstrable abnormality found by investigations on the hemorrhagic diathesis. Such reduced capillary resist-

ance, the origin of which remains obscure, was also demonstrated in some patients in whom there were no signs of hemorrhagic diathesis.

This hemorrhagic tendency in patients with poliomyelitis may manifest itself by bleeding from the skin or from the various mucous membranes. Hence it is justifiable to interpret intestinal hemorrhages merely as one manifestation of a general hemorrhagic diathesis and not necessarily due to a peptic ulcer. Besides no connection between hypertension and hemorrhagic tendencies has been observed.

Although patients suffering from acute poliomyelitis with respiratory paralysis are exposed to severe stress, the incidence of peptic ulcers must be considered low and there is no evidence that these patients are more apt to get a peptic ulcer than other people, irrespective of the degree of severity of their poliomyelitic sequel. This does not seem to be suggestive of a decisive importance of stress—at least not of brief stress—as regards the pathogenesis of peptic ulcers.

During the acute stage of the disease, paralytic ileus and gastric dilatation represent the most commonly encountered gastro-intestinal complications; as regards the latter, the dilatation caused by artificial ventilation cannot be excluded as a contributory factor.

During the chronic stage of the disease, constipation and fecal impaction represent the most frequent complications. The fecal impaction is occasionally severe but evidently causes no pains—probably because of the insufficient intestinal motility. However, the mechanical ileus represents an extremely rare complication at this stage of the disease.

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## Genito-Urinary Complications in Patients Severely Stricken by Poliomyelitis

DR AVRON Y SWEET

The sole urinary tract abnormality in acute poliomyelitis is the retention of urine which results from temporary paralysis of the urinary bladder. This small irksome problem plays an important role in the pathogenesis of urinary tract infections, calculus formation and in some cases ultimate renal failure during the post acute period in severely involved patients.

Most patients with severe poliomyelitis particularly those with involvement of the lower extremities have paralysis of the urinary bladder and many of these obtain relief with Furchthide, physostigmine or Urecholine. Those who are not helped must be catheterized. This act together with the continued presence of the catheter in the bladder is invariably responsible for infection of the bladder and in time ascending infection of the urinary tract.

Hypercalciuria is another important factor in the production of serious urologic complications in the postacute period in severely involved patients. It is commonly held that the flaccid paralysis and lack of weight bearing result in a diminution of protein matrix upon which calcium is normally deposited. Therefore the calcium which ordinarily would be deposited on that protein matrix remains in the blood and is ultimately excreted by the kidneys. The resulting hypercalciuria is usually in the magnitude of 3 to 4 times the normal daily excretion of calcium. Dunning and Plum have demonstrated hypercalciuria in patients with bulbar involvement only and suggest that it results from some central neuronal abnormality.

Bacterial infections of the urine play a villainous role in the production of complications of the urinary tract for regardless of the offending organism pus tends to increase the pH of the urine. Hypercalciuria and alkaline urine favor the precipitation of calcium in the urinary bladder rather early in the disease. Usually in crustations form about the indwelling catheter.

Later the same circumstances except for the presence of the catheter exist in the pelvis of the kidneys.

The incidence of urinary tract calculi apparently varies according to the degree and extent of paralysis but data are not available which clearly show this nor do we know the influence of respiratory abnormalities, duration of bed rest and prophylactic measures. An incidence of 48 per cent of all paralytic cases in adults was reported by Rodgers and his co-workers; however stones occur in 18 per cent of all patients who require respirators irrespective of the duration of respiratory assistance. The incidence of stones is much lower in children about 1 per cent. At the Poliomyelitis Respirator Center at the Mount Sinai Hospital in New York where patients have been admitted from 1 to 6 months following the onset of illness we have found the incidence of calculi of the urinary tract to be 40 per cent. We have not found any patient with stones to be free of infection. Neither has any patient with mildly involved or normal lower extremities irrespective of respiratory inadequacy, extent of paralysis elsewhere or duration of confinement to bed been found to have calculi.

With improved materials and techniques in care and rehabilitation an increase has occurred in the salvage rate and longevity of severely involved poliomyelitis victims. As a consequence the long term complications and intercurrent problems have attained great importance. The most frequently occurring difficulty is that of intercurrent respiratory infections. The next most common are the urinary tract diseases which include exacerbations of pyelitis and pyelonephritis, partial and complete obstructive uropathies, development of staghorn calculi and resultant diminution of renal function. Although respiratory infections are most frequent urologic complications are responsible for more

time lost from the standpoint of prolongation of hospital stay removal from active rehabilitation programs and readmissions to hospitals. In our experience urologic surgical procedures have outnumbered all other operations combined and are in general of a more serious nature. By 1948 the medical literature recorded but five respirator patients who had survived major surgery. Today major urologic surgery is not uncommonly performed upon severely involved respirator patients in whom postoperative complications are unusual and deaths are a rarity. In the main these procedures are ureterolithotomies, pyelolithotomies and combined pyelo-ureterolithotomies. Excellent results have been obtained because of experienced surgeons' careful choice and administration of anesthetic agents and meticulous postoperative care. Because of the lability of the blood pressure in respirator patients they must be positioned on the operating table slowly and gently while blood pressure determinations are made frequently. Anesthetic agents which are irritating to the respiratory tract are to be avoided. Ventilation is maintained through an endotracheal tube by manually squeezing an anesthesia bag or by an intermittent positive pressure machine. Usually surgery is complicated only by considerable oozing of blood which has been attributed to failure of the capillaries to contract because of perivascular fibrosis. Postoperatively careful control of respiration, attention to respiratory tract secretions and judicious fluid and electrolyte therapy are imperative.

The diagnosis of obstructive uropathy may be difficult for frequently severely involved patients do not complain of the prime symptoms of ureteral or ureteropelvic obstruction, namely pain. This has been attributed to involvement of the lateral columns of the spinal cord. The patient may complain only of abdominal discomfort caused by ileus. This occurs so frequently that we always obtain an excretory urogram when a patient complains of abdominal discomfort. Although patients with urinary tract calculi in our experience invariably have infection they often do not have fever associated with obstructive uropathy. Leukocytosis may or may not be present.

To prevent or minimize the deleterious effects of hypercalciuria and urinary tract infections attempts should be made to control these factors

during the acute phase of poliomyelitis. If catheterization of the bladder is done prophylactic antibiotic therapy should be given in therapeutic doses. Chloramphenicol is probably the most desirable drug inasmuch as it is effective against many of the gram-negative organisms as well as staphylococci. Irrigation of the bladder twice daily with Suby's solution may prevent the formation of bladder calculi. Removal of the bladder catheter at the earliest time possible is important. Fluid intake should be sufficient to produce a satisfactory daily urine volume. We have found that the administration of anabolic steroids, principally androgens, results in a 40 per cent diminution in the daily urinary loss of calcium in severely involved poliomyelitis patients. Therefore it would seem that androgen therapy might very well be added to the therapeutic regimen during the acute stage of poliomyelitis.

Following the acute phase of the illness androgen therapy should be continued, a satisfactory urinary volume should be maintained and if the bladder catheter has not been removed previously vigorous attempts should be made to do so. Antibiotic therapy should be continued until after the catheter has been removed and cultures of the urine are sterile. It has been suggested that rocking beds and tilt tables are beneficial in diminishing hypercalciuria; however, balance studies have shown that these modalities are useless. Rocking beds and tilt tables have been recommended also for the postural drainage of the upper urinary tracts. There is no evidence that this is necessary or that the devices used prevent stasis. Many authors urge the use of acid ash and low calcium diets while others suggest the administration of aluminum hydroxide gel in order to decrease phosphorus and calcium absorption from the bowel. There is no evidence to support the usefulness of these measures. Recently the use of salicylates for the prevention of calculi has been advocated, however its efficacy has not been substantiated.

There were no known disturbances in renal function associated with poliomyelitis until it was shown recently in our laboratory that hypercalciuria is accompanied by an increase in the renal excretion of sodium, potassium and chloride without alteration of the glomerular filtration rate. This abnormality played an important role in the death of one of our patients and it is

quite possible that it may be responsible for some of the chemical disturbances which occur during the acute and early postacute phases of poliomyelitis

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## DISCUSSION

Dr NEUKIRCH Dr Sweet's paper bears the title of genito-urinary complications in poliomyelitis but is concerned only with the urologic abnormalities. However the occurrence of complications involving the genitals should also be mentioned. During the acute stage of the disease menstruation disturbances are not infrequent and most—although not all—male chronic respirator patients are suffering from impotency.

The poliomyelitis virus per se does not directly affect the urinary organs and the diseases of the urinary tract occur secondarily to a series of complications and specific conditions involved in poliomyelitis which incidentally is not the only disease in which renal calculi occur. Not infrequently tuberculosis of the bones and joints will be combined with a calculus formation in the urinary tract a feature which generally is ascribed to the protracted immobilization and the consequent decalcification of the bones.

Figures from the epidemic in Copenhagen in 1952 (Table 178) serve to illustrate the incidence of urolithiasis in poliomyelitis. Calculi developed in 31 patients including 3 children among a total of 1,258 i.e. in 2.5 per cent of the cases among 345 respirator patients i.e. patients requiring artificial ventilation regardless of the duration of this ventilation calculi were demonstrated in 29 i.e. in 8.4 per cent of the cases. Among 26 chronic respirator patients i.e. patients who more than 12 months after the onset of the disease were still requiring assisted respiration calculi were demonstrated in 13 i.e. in 50 per cent of the cases. Among 31 patients with stone formation recorded in the table 29 had received respiratory assistance however calculi were also demonstrated in the 2 remaining patients although no respiratory paralysis was present. As opposed to information given in the paper of Dr Sweet 2 of our chronic respirator patients suffered a development of calculi although their lower extremities were not affected. Our material does not give any reliable evidence of relationship between the occurrence of calculi formation or the duration of paralysis of the bladder or the duration of catheterization. Several of our patients even

those with respiratory impairment in whom catheters were indwelling for several weeks and in whom an additional infection of the urinary tract occurred during the acute stage of the disease failed to give evidence of a stone formation. In 7 patients apparently the calculi disappeared spontaneously within periods of 7 to 3 years.

TABLE 178 POLIOMYELITIS IN COPENHAGEN 1952  
—URINARY COMPLICATIONS

	NO OF PTS	CALCULI PRESENT IN	PER CENT
Paralytic cases	1 258	31	5
Respiratory cases	345	29	8.4
Chronic respirator cases	26	13	50.0
Respiratory cases	all patients who for a longer or shorter period of time required respiratory assistance		
Chronic respiratory cases	patients who more than 12 months after the onset of the disease still require respiratory assistance		

Two patients with calculus formation—both requiring artificial ventilation—died from nephritis and uremia within 7 months and 3½ years respectively after the onset of the disease.

The role of artificial ventilation on calculus formation was only briefly outlined in the introduction. In particular the opinion was advocated that calculus formation might become accelerated through hyperventilation investigations indicate that the urinary pH increases the urine thus becoming alkaline during protracted artificial respiration but this feature is not a constant phenomenon. The role of artificial ventilation in the pathogenesis of the urinary tract concretions is still remaining obscure.

In our material calculi were surgically removed only on one occasion the result was poor and calculi soon reappeared. Other investigators found the surgical procedure successful particularly modern surgical and anesthetic



positive correlation between the degree of physical involvement and the emotional difficulties the patient has in adjusting to the disability. The emotional difficulties which are precipitated by the illness and residual paralysis will depend to a large extent upon the degree to which these interfere with the usually successful ways of satisfying emotional needs.

While the illness undoubtedly leaves its emotional scar on the developing personality of the child, the long term effects of the illness and its possible paralytic residual are less devastating to the infant personality when compared with older patients because the infant's self-concept usually has not had a chance to develop any significant internal consistence. Thus the person who has an early illness resulting in residual paralysis is frequently able to integrate such residuals into the developing personality structure with a minimum of difficulty. Adaptations are made by such an individual early in life by discovering and cultivating those things in which he is proficient resulting in adequate ego satisfaction. However the guilts and anxieties of the parents frequently become a major interference in the otherwise relatively effective adjustment of the ill child. Frequently the parents require the major efforts of the hospital staff in their adjustment to the disabled child.

The age of the patient is likewise of importance in understanding the emotional reaction to illness and the long term effects upon the personality structure. The infant will react to the physical trauma, the discomfort involved in the diagnostic procedures and the treatment program in a variety of ways—all the way from apparent indifference to stark panic. The infant has basic personality and emotional needs which the home has previously satisfied. These needs are frequently not met in a hospital setting thus causing bewilderment, fear and generalized panic in the patient. Children frequently find it difficult to understand why they cannot be at home, why their mothers cannot care for them and why they have been abandoned in a strange place with strange people. The emotionally barren hospital environment can be minimized to some extent if the personnel caring for the patient give affection in addition to the physical care. It may be presumed that those infants and children who come from an emotionally secure home environment will experience less threat

in the course of their illness and its sequela than those coming from emotionally inadequate homes.

Adolescents who become ill and are left with paralytic involvement have a greater adjustment problem than probably any other age group. In adolescence the self-concept is undergoing further rapid differentiation and refinement. It is in this age period that the personality structure becomes relatively well crystallized resulting in the establishment of tentative life goals. Relationships with others of the same age become of primary importance resulting in heightened sensitivity to the approval or disapproval of the group. It has been noted that adolescents who have received their major ego satisfaction through the function of the physique have greater emotional difficulties when left with a residual paralysis than those who depend primarily on nonphysical means for such satisfaction.

Adults react to their illness in various ways these being determined by psychodynamics similar to those of the younger age groups. In dealing with the emotional reactions of the adult patient we must try to understand how the illness and its residuals affect his ego needs. Most patients become emotionally disturbed because they anticipate that their illness may make the pre-illness life role impossible. The patient may need to face the problem of how the illness will affect his role as a parent, spouse and sex partner.

There is a difference in the variety and intensity of emotional reactions of poliomyelitis patients. Some of these emotional reactions have been clinically and experimentally studied. One of our investigations has indicated differences with regard to the age of the patient as well as indicating that male and female patients 7 years of age and above tend to admit to more emotional turmoil at or soon after onset while in patients below this age there tends to be a delay of 6 to 8 months in this regard.

There likewise appear to be some differences between males and females in the emotional reaction to varying periods of hospitalization. During the first 6 months after onset most patients express the feeling that they will make a complete recovery. This is probably the only adequate available psychological defense which is able to temper the traumatic significance of

the illness and its paralytic residual. We find that our adult male patients have greater definable anxiety than do the female patients. Male patients tend to suppress more of their anxiety than do female patients who may drain off more of their feelings by acting out behavior such as weeping and being unusually demanding of the nursing staff. Male patients as a group appear to reach peak emotional tension somewhere between 4 and 6 months after onset while in the females this tends to appear somewhere between 7 to 9 months.

The emotional factors which accompany this illness and the necessary long term hospitalization would have a dampening or inhibiting effect on the effective use of the patient's intellectual faculties. Recently the verbal portion of the Wechsler Adult Intelligence Scale was administered to 93 patients of whom 71 were in patients at the time of testing. On a purely descriptive level we found that the average intelligence score of the 46 males tested was 113 and the average score of the 47 females tested was 106 with an over all average score of 109. Two other trends are noted in our sample: (1) patients tested soon after admission to the hospital tested consistently lower than those who were tested some months or years after onset; (2) the patients who were in the younger age groups tested lower than those who were older in age.

Another factor which affects the emotional reaction of the patient is the rate of recovery. That is patients who make early steady tangible progress in their physical rehabilitation program appear to be less emotionally disturbed than patients who make no progress or whose progress is interfered with by secondary medical complications.

Further emotional turmoil revolves around the effect the illness has or is felt by the patient to have on the family. How the family reacts to and with the patient during the early stages after the onset of the illness has emotional significance for the patient. The patient's illness is an emotional trauma to the whole family. Patients of all ages frequently feel that now that they have lost much of their function and may become incapacitated they no longer are lovable or acceptable. Such feeling reflects the emotional significance of the illness to the patient.

A number of psychological reactions are clini-

cally observable during the hospitalization of the poliomyelitic patient. During the initial stages of the disease most patients demonstrate feelings of anxiety which are verbalized and sometimes acted out in a variety of ways. Some of the anxiety symptoms are related to hyperventilation which are expressed as feelings of tension and loss of emotional control or even consciousness. The expression of anxiety follows a somewhat cyclic course with acute anxiety being followed by a reduction and again an intensification. The characteristics of these cycles are of an individual nature and perhaps parallel closely the stages of physical recovery. The anxiety which we see in most of the patients is of a reactive type with the bulk of the anxiety being consciously or unconsciously related to the disease and recovery process.

Another form of behavior frequently seen in the respiratory poliomyelitic patient can be described as regressive. When the majority of a patient's time is taken up with treatment of physical and emotional problems and when he is constantly reminded of the immediate pain involved in the illness it is understandable that his behavior should be less mature and more narcissistic than prior to his illness. The patient who has bulbospinal poliomyelitis resulting in muscular paralysis requires 4 hours a day care during the initial stages of the disease. Perhaps such intensive care reactivates unconscious patterns of infantile behavior. The regressive behavior patterns usually disappear when the patient begins to regain some function making independent movement and breathing possible. Patients who are very severely involved orthopedically and are in the respirator full time frequently exhibit regressive behavior over a period of months and years.

Many of the patients become depressed during the course of their hospitalization. The paralysis of the body structure with the subsequent changes which the patient may need to make in his self-concept may cause feelings of depression. The loss of those aspects of the personality structure produce feelings similar to grief or sorrow since it is in a sense the death of a part of the pre-illness personality. Our experience with patients in a reactive depression would indicate that this is usually a healthy sign in that it indicates that the patient is coming to grips with the reality of what the

disease and its residual paralysis means. The depression is in a sense a period of mourning for the death of the old self. Again patients who make rapid and extensive physical progress in their recovery usually are not as depressed as those who do not make such progress but psychodynamically speaking the patient who makes such progress does not need to be as depressed since the effects of the illness may not necessitate a major personality reorganization. The patient who steadfastly insists that his body has not changed or that he has not had poliomyelitis or that he is not paralyzed may not go through a period of depression because he denies that which in reality has happened to him.

Hostility is an emotional reaction frequently seen in the bulbospinal poliomyelitic patient. A minimum of hostility is expressed during the initial stages of the disease. However as the course of hospitalization continues hostile feelings tend to be expressed. The expression of these feelings it is believed indicates that the ego-strength of the person is in the process of redevelopment. The expression of negative as well as positive feelings occurs for several reasons. The expression of legitimate feelings may help the patient determine how acceptable he is as a person. If his feelings though they are of a negative sort are accepted it will make him feel that he as a person is acceptable. The first hostile feelings are frequently directed toward the self. The patient frequently blames himself for having become ill. He may explain it on the basis that he was working too hard or that he had sinned and God is punishing him or in

some other way attempt to explain his illness to himself. It is probably necessary for many patients to go through a period of self-condemnation as a means of reducing their own guilt feelings about being ill and possibly a burden on others for the rest of their lives. It does not take too much ego-strength to express feelings of this sort and since such feelings do not threaten others frequently not too much attention is paid to them.

More specific hostile feelings are often expressed. These may be directed at the doctor who first saw them. This sort of hostility takes more ego-strength and while the validity of what is said may be questioned the significance of the feelings cannot be ignored.

Many patients at some time or other express hostile feelings directly to the nurse, the therapist or some other member of the hospital staff. The patient may condemn the therapist because he has not made a better physical recovery. The patient may project some of the responsibility for the illness and residual upon the parents, husband or wife.

The responsibility of helping the patient with his emotional feelings cannot be assigned to any specific member of the staff but remains a mutual responsibility of the whole team. We must accept the feelings of patients in the same way we accept the variety of physical problems which accompany the patient's physical course. The Respiratory Center is interested in the whole person and provisions must be made to deal effectively with all problems related to the patient's rehabilitation. To facilitate the patient's total rehabilitation the emotional problems must be dealt with effectively.

## DISCUSSION

**PROF. DR. HOLST:** What do you know about the mental problems of your fellow men?

Often we will not encumber even our next of kin with all our mental problems—either because they ought not to be disclosed or are so difficult that we are unable to define them. Questions of death and God will certainly represent mental problems to most thinking men—yet few of us want to make these questions objects of earnest discussion. Most persons do not want to discuss their mental problems if they are ill and personally I never try to probe the minds of dying persons. But under less crucial circumstances one of the doctor's most important tasks is to obtain the confidence of his patients in order to relieve them if possible of some of those problems which may hurt them and retard their rehabilitation.

When very small children are taken ill with polio they have no mental problems because they are not able to create mental problems. Unless the individual has reached a certain stage of maturity which is not dependent upon physical age he will not be able to create mental problems even if he is fully able to register impressions of pain.

Knowledge of polio is so common that we must expect fright to be the first reaction in a thinking person. He will have heard of other patients who have died from the disease or spent years in a respirator or been crippled for the rest of their lives. In some cases the fright will be well founded. The patient will suffer from respiratory insufficiency and under these circumstances his mind will hardly have room for more than one thought—how to obtain enough air in order to evade suffocation. Not is the origin of much fright and anguish. It is like living in a world where time and air will stand still and tremble. Not until respiratory insufficiency has been relieved will conscious-ness be penetrated by other thoughts. These will mainly concern questions of life and death. If I live what will be the result? If my hour has come what then? These questions will partly be answered in the course of the first 2 weeks. Patients still living after that time may look

forward to an indefinite continuation of life. But even now there will not be much occasion for reflection. The patient will not have adapted himself to his new and cramped position in the tank cuirass or positive pressure apparatus. After a recent tracheotomy he will certainly have troubles from his airways. Suction must be applied, roentgenograms and bronchoscopy will often be necessary. He must be tended like a baby. Unknown persons will come and go. His life will be full of new and mostly painful sensations. In fact there will be neither time nor opportunity for reflections beyond the most pressing physical needs.

But by and by the patient will realize that there may be a future even for him. He will have heard about other cases which have completely recovered. His pessimism will give room to optimism and impatience. He wants to get out, wants to start physical exercise which represents the road to restitution. At this time he will have no conception of what will be demanded from him of courage, toil and endurance perhaps for the rest of his life.

Naturally most of our young parents pity themselves and worry because they are wasting their youth. If they have no financial liabilities they will certainly have smaller problems than those who are responsible for a family. In these comparatively lucky cases life will be shaped by the degree of invalidity and by impressions from the surroundings.

Mental problems will be colored by treatment and improvement. Having lost his self-confidence the patient will often energetically oppose any proposal for taking physical exercise which he thinks may hurt him or punish him. He will be afraid of breathing with his own muscles or bathing in the Hubbard tank or of having his tracheotomy tube removed etc. His physical inferiority complex will take a long time to be conquered.

His mental problems will be intimately concerned with his fellow patients and the nursing staff. His egocentricity will often take the form of jealousy and this may lead to personal antipathies but also can have the opposite effect.

the patient will vehemently oppose any change in the nursing staff

A temperamental crippled young lady writes

One of the greatest difficulties is how to regain your self-control when you have lost your ordinary means of expression like banging the table and slamming the door. It is also very very difficult always to be grateful in a really gracious way.

Married people will have more serious mental problems. The economic problems will certainly make themselves felt. How can the family exist if the breadwinner is taken away or if the working capacity of his wife is so reduced that she must have a substitute? The latter eventuality is often worse than the former. We can generally find financial support for a family which has lost the breadwinner but it is difficult to find a substitute for his wife. This will cost so much money that the ordinary wage earner cannot afford it and apart from the financial question it is practically impossible to find a capable woman who is willing to tackle a job like that.

What about the education of the children if one of the parents is forced to inactivity? If this question has not been given thorough con-

sideration it certainly will be emphasized during idle hours—before sleep sets in. Parents will ponder the hundreds of things which they may want to teach the children opportunities and possibilities which may be lost forever.

What about the healthy partner? Will he or she be able to withstand the stress of the situation? Eventually will he or she maintain his or her feelings toward the patient? Experience has taught that the patient may indeed be doubtful on this issue.

Mental problems will be many and varied according to environment and time. Sex and religion ought to be discussed apart.

We must remember always that the virus does not destroy either the sexual glands or the brain. The crippled patient will keep his hormonal impulses and his daydreams. This may lead to a tragic result.

In some patients the malady may create a dull apathy but lack of bodily activity may also stimulate mental activity. The patient will meditate upon the meaning of life and the lucky ones may find solace in religion. This certainly will be a great help for those who try to learn that most difficult of arts—the art of resignation.

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# Care of Patients Severely Stricken by Poliomyelitis

FRIDAY MORNING, JULY 12, 1957

(This Session Convened in the Aula of the University of Geneva)

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# *Neuromuscular Factors in Rehabilitation After Poliomyelitis*

DR FRANK S COOKSEY

Until recently the management of muscles paralyzed by poliomyelitis consisted of general and local rest in the active stage of the disease followed by progressive exercise of the affected muscles during convalescence. Rest including the use of rigid splinting often was prolonged because it was thought necessary to prevent weak muscles being fatigued or stretched by the unopposed action of gravity or normal muscles. Remedial exercises were graduated carefully to avoid fatigue and localized to weak muscles. Trick movements and functional adaptation were discouraged until a prolonged course of remedial exercises had failed to restore normal muscle anatomy.

During the past decade or so developments in neurophysiology and clinical practice have led to significant changes in the management of paralytic poliomyelitis. At present there is general agreement that rehabilitation after poliomyelitis can be started earlier and carried further than has been customary in the past but there are still controversial views on when remedial exercise should begin and how vigorously it should be pressed. Much work has been done and the literature is extensive. Even so there is still much to be learned about the nature of muscle fatigue and the factors concerned in the atrophy and recovery of muscles.

## REST AND ACTIVITY

The long-established value of general and local rest in limiting the spread and aiding the resolution of any infection is still undisputed. In poliomyelitis, in particular, there is evidence that rest in bed in the prodromal stage and while the infection is still spreading is a significant factor in limiting the incidence of paralysis. Nevertheless like most therapeutic agents rest has adverse side-effects about which much more has been learned in recent years leading to important changes in many aspects of clinical practice. For instance early ambulation after surgical operations and in the management of the

degenerative disorders of the elderly can be cited as examples.

Mainly due to the advent of antibiotics it has become possible to restrict the use of rest in the presence of infection. This has emphasized the adverse effects of rest which were formerly dismissed as being of secondary importance to the control of infection. In the absence of an antibiotic for poliomyelitis rest continues to be of major importance until this infection has subsided. Nevertheless even in the acute stage a balance can be struck between the beneficial and adverse effects of rest.

Many workers have drawn attention to the severe wasting which occurs in normal muscles when they are immobilized especially when immobilized in the shortened position. Similar wasting occurs in muscles involved in peripheral nerve injuries and in poliomyelitis. This wasting was attributed to reduction in blood flow consequent upon inactivity but it now seems to be recognized that the maintenance of tone in normal muscles is mainly dependent upon the proprioceptive stimuli developed in the muscle spindles during movement. Wasting and reduction in blood flow follow when these stimuli are reduced by immobilization or by interruption of the afferent or efferent neurons. In experimental animals the effect of immobilizing normal muscles in the stretched or shortened position is to cause as much wasting as when the anterior or posterior roots are divided although in recent work it was found that the blood flow had actually increased.

A feature of poliomyelitis is that stiffness and contracture of muscles may set in early and insidiously lead to intractable deformities. Immobilization tends to increase the contractures while passive movement helps to prevent or overcome them.

Less is known about the effect of rest and activity on the central nervous system. Some workers hold that the transmission of stimuli by synapses when inflamed by the virus is

detrimental to them. Others point out that synapses are so sensitive to anoxia caused by inflammatory edema that they do not respond to stimulation until recovery is well advanced when no harm ensues. Nerve cells which survive the infection appear to recover in a few weeks so that even if rest is necessary in the acute stage it need not extend beyond early convalescence.

Based on the foregoing the present trend in the management of the acute stage is to rely on general rest in bed to combat the infection and to employ passive movements from the onset in order to maintain some proprioceptive stimulation to aid the circulation in the muscles and to prevent contractures. Gentle stretching of weak or paralyzed muscles during passive or active assisted movement is now thought to be beneficial rather than the reverse. At rest muscles are maintained as far as possible in the neutral position by means of pillows or sandbags and the use of rigid splints or plaster casts is avoided except when necessary to oppose the action of gravity as for example with opponents pollicis.

When the infection has subsided and convalescence has been established for some 3 or 4 weeks unaffected muscles are exercised to prevent further wasting and weak muscles started on graduated exercises. At this stage two points need to be considered. First the effect of fatigue on weak muscles. Second whether to exercise individual weak muscles or to develop them together with other affected or normal muscles in their functional movement patterns.

One of the controversial problems is the effect of fatigue on muscles recovering from poliomyelitis. In the past fatigue was thought to be harmful and great care was taken to avoid it. Recently Ritchie Russell has stated that as fatigue is the physiologic stimulus to muscle hypertrophy remedial exercises should be graded to produce it if the maximum benefit is to be obtained. In between those who say avoid fatigue and those who say push muscles to fatigue are those who hold that fatigue in moderation does no harm but that it is not necessary to fatigue muscles in order to increase their strength.

Fatigue may be due to muscular effort exceeding the available blood supply so that metabolites accumulate faster than they can be removed. However the commonest cause of fa-

tigue in muscles is fatigue in the corresponding synapses of the nervous system. This applies both to synapses concerned in the direct motor supply to a particular muscle and in the associated pathways which co-ordinate its action in a functional movement pattern. Synapses are very sensitive to disuse and respond rapidly to exercise. There is no convincing evidence that synapses are harmed by being fatigued.

There is a difference between physiologic fatigue and excessive exercise leading to muscle exhaustion. The latter may cause marked swelling followed by ischemia and necrosis in muscles which are confined by aponeurosis such as the anterior tibial group. This effect is easily produced in partially denervated muscles and care must be taken to avoid it.

### THE RE EDUCATION OF WEAK MUSCLES

In the early stages of convalescence there is spontaneous recovery of those motor units whose anterior horn cells have been put out of action temporarily but not destroyed. While the recovery of function is spontaneous up to a point the disuse atrophy in the muscles and in the synapses of the central nervous system will persist until reversed by progressive activity. That is to say rehabilitation is necessary even for muscles which recover from an attack of poliomyelitis if full function is to be restored without undue delay. One so often sees patients who make good recovery with practically no weak muscles but then everyone thinks there is no need to do anything about rehabilitating them. Of course there is the same need to rehabilitate those patients as anyone else who sustains severe illness.

Recent work on the integrated activity of the central nervous system and the importance of the proprioceptors in the muscle spindles as the focal points in the control of movement have led to the development of new techniques of re education through functional movement patterns in preference to training individual weak muscles. This important advance in kinesiology has greatly facilitated rehabilitation after poliomyelitis as well as in other disorders of the locomotor system.

Nevertheless in poliomyelitis in particular there is still a place for the training of individual muscles especially in the early stages. Muscles



which lose motor units through the destruction of anterior horn cells have to compensate by reorientation and hypertrophy of the surviving motor units within the affected muscles. Thereupon the central nervous system will tend either to modify the movement patterns in which the affected muscles are concerned so as to make the best functional use of what remains or to substitute other normal muscles if available for the weak ones which will then atrophy from disuse. The weaker the muscles the greater will be the tendency to substitution and consequently the lesser the likelihood of the affected muscles being strengthened by being used. Therefore it is essential to exercise individual weak muscles to obtain the maximum hypertrophy of surviving muscle fibers in order to avoid unnecessary substitution.

However the training of individual weak muscles will not restore functional activity as a whole. If a weak muscle is exercised in isolation fatigue at first sets in rapidly but diminishes with facilitation. Yet if the muscle is now exercised in its normal movement pattern it will again fatigue because of lack of facilitation in the mechanism of associated movement. Therefore the training of individual weak muscles must be combined with re-education through functional movement patterns.

Exercising muscles to the point of fatigue against increasing loads improves their power, i.e. the maximum tension which they can exert. Facilitation of the synapses in the central nervous system through practice increases the speed co-ordination and endurance of muscle action. Accordingly the achievement of full rehabilitation without undue delay necessitates a program of remedial exercises and occupational therapy starting as early as possible and calling for increasing effort by the patient for longer periods each day until the maximum response has been obtained. That is to say one must provide decreasing effort against load and increasing practice to obtain co-ordination and endurance and speed. A recent study of two

groups of cases subjected to intensive rehabilitation, one starting at 4 weeks from the onset and the other at 8 weeks has shown a similar degree of improvement in the affected muscles and no untoward effects in the group which started at 4 weeks.

Poliomyelitis is an extremely variable disorder and clinical judgment must be relied upon in the management of individual cases. Nevertheless the recent work does seem to suggest that in general rehabilitation after paralytic poliomyelitis can safely commence 1 month from the onset and be pressed more vigorously than has been customary in the past.

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## DISCUSSION

Dr SHARRARD I would like to endorse much of what Dr Cooksey has said in his careful resume of current trends and thoughts on the treatment of paralyzed patients

During the past 50 years all manner of treatments have been used many of them based on accepted custom ignorance prejudice or misapplication of facts rather than on an appreciation of the pathology physiology and clinical progress of the disease What Dr Cooksey has said this morning shows that we are now much more appreciative of these things

General rest during the acute stage is vital to minimize the spread and activity of the virus in fact 1 or 2 weeks of bed rest may not be enough because a relapse with serious consequences can occur as late as the third week Vigorous active exercises during the first 3 weeks also can cause further spread of the paralysis which should be a warning to those who are overenthusiastic that there is a lower limit to the time at which an active program should begin

However passive joint movements and muscle stretching are safe and are essential to prevent contractures Weak or paralyzed muscles are no more susceptible to stretching than normal muscles On the contrary excessive relaxation of muscles is likely to discourage recovery as the following results show Of 81 completely paralyzed muscles treated on abduction frames for periods up to 2 years 17 recovered of 93 completely paralyzed muscles that were treated on no abduction frames 35 recovered

### TREATMENT IN THE CONVALESCENT STAGE

It is not appreciated generally that spontaneous recovery of intact but inactive motor units though not definite during the first 6 weeks may still continue up to the fourth month The motor cells in this photograph (Fig 334) were all that remained of the nucleus of the quadriceps muscle all the cells there were in a patient who died suddenly at the end of the fourth month He had been receiving active physiotherapy treatment yet the quadriceps had only

started to show a flicker of contraction 2 weeks before he died in other words those cells had been in abeyance for 4 months Note the vascularity and cellular infiltration of the supporting tissues 6 months after the disease Edema which is a prominent feature does not finally subside until the eighteenth month

While it is doubtful whether exercises or the lack of them make any difference to the intrinsic recovery of the neuron I agree with Dr Cooksey that the resumption of muscle action needs active effort and that this is probably better achieved by the training of individual muscles or muscle groups than by the use of functional movement patterns in the early weeks This program of exercise can well commence at the fourth week



FIG 334 Gray matter of spinal cord 4 months after acute anterior poliomyelitis Two motor nerve cells constitute all that remains of the nucleus of the quadriceps muscle The muscle showed clinical contraction before death and a streak of innervated fibers was found in the muscle at postmortem Note the vascularity and cellular infiltration (Proceedings of the Second International Congress of Neuropathology London 1955)

After the second or third month it is logical that training in movement patterns should be introduced and gradually predominate over individual muscle exercises in preparation for a return to functional activity.

I mention electrical stimulation of muscle or nerve only to condemn it. Spectacular as it may be to a patient to see his paralyzed muscles acting under its influence there is no physiologic basis for its use: it wastes time, discourages voluntary activity, and in clinical trials does not accelerate or increase the amount of recovery.

Concerning fatigue and exercises while I accept that fatigue defined as a falling off in power of a muscle that is being exercised is not harmful should it occur I cannot believe that fatigue should be the aim of treatment.

An intensive exercise program too is impracticable in a patient with many weak muscle groups. If as has been suggested a muscle group should be exercised for 5 minutes every hour then it is only possible to treat 9 or 10 muscles or muscle groups in this way.

However if a muscle is to become more efficient it must be made to work hard. In my experience a regimen in which muscles are exercised up to the point of fatigue and all weak muscles are given an equal amount of attention during the day achieves as much as a more intensive method. In support of this I show the results of an analysis of muscle power (Table 179) after the completion of such a program just to demonstrate that with such a program

muscles which are grade 3 need only to have 5 to 10 per cent of residual motor cells and a muscle which is grade 5 that is normal may lose as much as 60 per cent of its nerve cells and still work as a clinically normal muscle.

PROF. DR. WALTHER. First I would congratulate Dr. Cooksey for his excellent communication. I quite agree with his view that it is indispensable if one does not want to lose or waste precious time or even jeopardize the future of the poliomyelitic patient to start with muscular exercise either by passive movements wherever possible or by active kinesiotherapy as quickly as possible after the acute phase of the disease. In this direction I even go further than my colleague. There are indeed cases in which the general state of health has not been seriously affected. I have adopted as a working principle the procedure of undertaking muscular re-education as soon as the patient can actively collaborate in the exercise.

When the patient has returned to what I call the normal vegetated balance that is if he no longer has any fever and he sleeps and eats well then he must not be considered a diseased person or an unhealthy person but an invalid with good general health. Disease and invalidity are not synonymous. If the person is sick then every effort must be avoided because a sick person needs all his reserves of strength to struggle against the disease whereas the invalid is an individual in whom the state of health is re-established or recovered but who still has lesions in this case of the locomotor nervous system and therefore of the muscular system.

In regard to the intensity of physical exercise it might not be superfluous to remind that the therapist must take into account the general state of health of the patient. Just as in sports training must be undertaken carefully and adapted to each case. If the patient has had to remain in bed and with complete rest we must not now go to the other extreme and overburden the recovering patient by a too intensified training. It is important in this respect to observe for instance the muscular reactions after repeated stimulation with galvanic current. As the electric stimulation sessions are prolonged contraction of a paralyzed muscle group will decrease in correlation with the intensity of the applied current. In other words muscular or synaptic fatigue

TABLE 179 THE RELATIONSHIP BETWEEN  
MUSCLE POWER AND RESIDUAL MOTOR CELLS  
IN THE SPINAL CORD

MUSCLE POWER (MRC SCALE)	PERCENTAGE OF RESIDUAL MOTOR CELLS
0	0-2
1	2-3
2	3-5
3	5-10
4	10-20
4	20-40
5	over 40

The figures were obtained by counting. If the remaining motor cell in each muscle and a firm complete serial sections of the spinal cord and comparing them with the known clinical power of the muscle before death.

turns up much more quickly than in normal muscles. One should not prolong electric stimulation sessions beyond approximately 10 minutes.

What has been observed during electric treatment also can be seen in medical gymnastics sessions. We should not ask the patient to make too much of an effort at the beginning of the treatment. The readaptation can be continued over years but as with other persons the poliomyelitic person has a need for holidays. Therefore the treatment should be varied and differentiated as much as possible and if pursued over long consecutive months should be interrupted completely and frequently. We have been able

to observe that an interruption of every treatment during the holidays under another climate for instance in the mountains of Switzerland helps the patient considerably. After a change of environment and of climate the paralyzed muscles had spontaneously recovered some vigor and the poliomyelitic patient could much more easily tackle his medical gymnastics. The holidays the stay in the higher mountains for instance made it possible for him to accumulate new reserves of strength reserves which he needed for future readaptation and from which the neuromuscular system had benefited in the first place.

# Prognostic Implications of Electromyography in Poliomyelitis

PROF DR FRITZ BUCHTHAL

This report reviews the evidence as to the extent to which electromyography in the early stages of poliomyelitis allows the final degree of paresis to be predicted. Such a study requires investigation both by electromyography and by grading of muscle force within the first month after the onset of the disease and reinvestigation, if possible repeatedly but in any case 1 year later.

What aspects of the electromyographic pattern are to be considered in such a comparative study? First is the general pattern of electrical activity of the muscles during maximum voluntary effort. This ranges from the so-called interference pattern of normal muscle with the simultaneous activity of numerous motor units to the pattern seen in severe neurogenic paresis where the activity recorded is of single motor units. This range of electromyographic patterns agrees fairly well with the grade of muscle force as estimated by experienced physiotherapists (Fig 335). The abscissa is muscle grading and the ordinate is a pattern of electromyographies: the lowest single oscillations and above interference patterns indicating the activity of many motor units. At 1 year after the onset of the disease the degree of electrical activity corresponds to a relatively higher degree of muscle force than at the onset. Evidence exists that this discrepancy is not due solely to the inability of acutely involved painful muscles to work at their potential maximum. Rather it may be due to an increase in later stages of the disease in the size

of the motor units by secondary branching of the nerve fibers to include denervated muscle fibers.

Then there are the characteristics of the single motor unit potential: duration, amplitude and wave form. Finally there is synchronism, the interrelation of discharges in different regions of the same muscle to a degree exceeding that in a corresponding normal muscle.

In addition to these characteristics of the electromyogram during voluntary effort there is the electromyographic picture at rest. There is no electrical activity in relaxed normal muscle but 2 to 4 weeks after partial or total denervation spontaneous discharges occur which have a much shorter duration than the ordinary motor unit potentials.

Comparative studies of the electromyogram and muscle force in paretic muscle during the acute stages of poliomyelitis have been carried out in Copenhagen beginning in 1942 and this series of patients and 2 subsequent ones have been in agreement that there are very definite correlations between the early electromyographic findings and the final degree of muscle paresis. The most recent series of patients was the largest and the most carefully controlled: 43 children were studied at the Boston Children's Hospital in 1952 and were re-examined in 1953. The findings are shown briefly in Table 180.

To estimate the prognostic value of action potential analysis the patients were grouped according to the degree of initial paresis and of

TABLE 180 GROUPING OF PATIENTS

GROUP	STATE OF MUSCLE WHEN EXAMINED		RECOVERY
	AT ONSET	AT 1 YEAR	
R-	Zero to fair	Zero to fair	Zero to slight
A	Fair to good+	Fair to good+	Zero to slight
R+	Zero to fair	Fair+ to normal	Large
N	Good to normal	Good to normal	Zero to slight

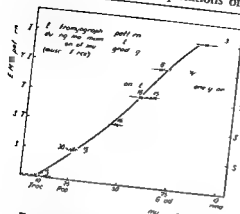


FIG 335 Electromyographic patterns during maximum effort as a function of muscle grading

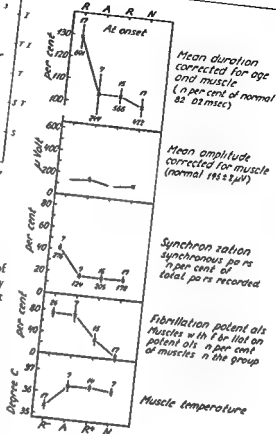


FIG 336 Patients grouped according to the degree of initial paresis and of recovery from it 1 year later

recovery from it one year later. On account of shortage of time I beg you to pay attention only to the group nominated R— a group without recovery and the equally paretic group R+ with recovery 1 year after the onset of the disease. That is to say that the 2 groups had muscles which were equally paretic at the onset but the plus group showed greater improvement in the course of 1 year.

The main results are shown in Figure 336 and again on account of shortage of time I beg you to look only on the left column and again on the group R— and the group R+.

Let us begin with action potential duration. The mean action potential duration obtained at a constant intramuscular temperature corrected for the age of the patient and for the muscle investigated was in the acute stage of the disease 30 per cent increased in severely affected muscles without signs of recovery 1 year later (group R—). Severely paretic muscles which improved greatly in the course of 1 year had normal values of mean action potential duration. In paretic muscle of all groups the incidence of polyphasic potentials was double that in normal muscle without regard to the initial degree of involvement or the final recovery. The increase in polyphasic potentials thus cannot account for the increase in mean potential duration in the R— group. Mean action potential amplitude was not correlated with recovery.

The incidence of synchronous activity between different leads in the same muscle was

clearly correlated with the degree of recovery. Synchronism occurred in 40 per cent of paired leads in severely paretic muscle without recovery (R—) against 20 per cent in the other groups and in normal controls. In the majority of the cases synchronization occurred within 10 days after the appearance of the paresis in some cases as early as 1 day after the clinical manifestation of paresis. A synchronization still present after the twentieth day persisted more than 6 months or 1 year after the paresis and was found in muscles with no recovery.

In paretic muscles with no recovery spontaneous low voltage activity of short duration (fibrillation potentials) occurs in a much higher percentage than in muscles with good recovery. The difference was 75 per cent against 33 per

cent. There was no spontaneous motor unit activity at rest in the cases which we have seen. There is no evidence of spasm in affected muscles either initially or after recovery.

An incidental finding (as indicated in the last column) was that the intramuscular temperature was correlated to the degree of involvement and recovery in that severely affected muscles without recovery showed a significant decrease as compared in temperature about 1 degree as compared with other groups and normals.

The finding of a correlation between muscle action potential duration and synchronism in the acute stage of poliomyelitis on the one hand and degree of recovery as determined by muscle force grading on the other indicates that the eventual outcome of the muscle paresis is largely determined by the initial disease process. Early electromyography thus may provide a guide as to which muscle will and which will not benefit by physical therapy directed to those muscles.

As to the cause of the electromyographic changes in the paretic muscles in the acute stage of poliomyelitis the electromyographic pattern at maximum effort is readily explained by the loss of many motor units which in the later stages corresponds to the histologic picture of scattered islands of normal tissue on the background of degenerated muscle. Recently it has been shown that the duration of motor unit potentials in normal muscle depends on the spatial distribution of the innervation zone of those muscle fibers of the motor unit which contribute to the motor unit potential. Thus it is most likely that the increase in action potential duration in muscles paretic from poliomyelitis is due to an unusually wide innervation zone; this might occur if the motor unit undergoes an enlargement such as could be brought about by the simultaneous firing of more than one of the surviving ganglion cells. A simultaneous firing would not only give rise to a pseudo-enlargement of the motor unit but also would evidence itself as a synchrony in different re-

gions of the muscles. Recent multi-electrode studies on the area occupied by the fibers of a motor unit have shown that this may be up to four times as large in severely affected paretic muscles as in normal muscle; the area exceeding even that of the largest motor units in normal muscle. Thus survival of the largest motor units cannot account for the average increase in potential duration in the early stage of the disease. Another possible cause of the increase in action potential duration is a decrease in the velocity with which the action potential is propagated over the individual fibers; this has been invalidated by recent measurements of the propagation velocity over severely affected muscles.

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**DR JASPER** Our experience with the use of electromyograph for prognosis in poliomyelitis is based largely upon examinations carried out with Mrs. Gwen Ballem of 50 patients stricken during the epidemic in Montreal in 1946. We examined 35 of these during the first 2 or 3 weeks following the onset of their illness and followed 25 with re-examinations from 9 months to more than 1 year.

Our results insofar as we have repeated the simple procedures have been in general confirmation of those presented by Dr. Buchthal with a few additional findings which I present for discussion.

I was surprised to hear that Dr. Buchthal and his associates did not find any evidence for spasm or spontaneous motor unit activity during the initial stages of the disease. In a number of our patients spasm was a prominent feature of the disease during the first week or 10 days. In some this was of sufficient severity to threaten flexion deformities of the limbs and it was painful. It was a reflex spasm since it could be made to disappear almost entirely in most cases by posturing the limb sufficiently to eliminate any stretch on the muscles. In 2 patients with the assistance of an experienced anesthetist the spasm was abolished completely by blocking of the dorsal roots with 5 per cent procaine. This produced a sensory anesthesia of the affected limbs without a motor paralysis. The muscles relaxed completely even with full extension of the limbs and voluntary movement was preserved. The involved muscles were then silent at rest with no fibrillation or motor unit discharge except on voluntary movement. However, although the presence of spasm was of importance during the early stages of the management of these cases it did not seem to imply a poor prognosis for eventual paralysis unless it was succeeded by a flaccid paralysis and fibrillation or permanent electrical silence in the electromyogram.

Another observation not mentioned by Dr. Buchthal caused considerable concern. This is the electrically silent muscle showing no fibrillation or motor unit activity even during

maximum voluntary effort. We have found such muscles in some patients even during the acute stages of the disease with no recovery and severe atrophy found on re-examination 9 months or 1 year later. One such patient was examined on 4 occasions during a year and we were never able to detect any electrical activity in the completely paralyzed quadriceps from the beginning. Neither were we able to produce a muscle contraction by direct electrical stimulation. Such findings though not common carried with them the poorest prognosis more severe than the fibrillating muscle. We were under the impression that such results must imply an involvement of the muscle tissue itself by the disease process since no such results have been seen in extensive studies of traumatic lesions of peripheral nerves except when a severe ischemia of the muscle has been added as well.

Interpretation of an electrically silent muscle is not always easy unless combined with studies of indirect and direct electrical stimulation. We have found in all cases that the interpretation of electromyographic results is greatly improved if combined with the results of electrical stimulation. In a few cases we have found an electrically silent muscle which responded well to electrical stimulation and to reflex excitation and yet the patient was unable to move it voluntarily. Hysterical paralysis is always considered in such cases but in a few instances we felt that there was a genuine organic difficulty in the voluntary innervation of muscles not necessarily involving the peripheral muscular system.

Also there is the interpretation of the polyphasic action potential of long duration. There are those which occur early in the disease similar to those seen in progressive neuromuscular disease like progressive muscular atrophy which must have a different interpretation than those occurring late. In the later stages the interpretation of Dr. Buchthal seems reasonable that there is extensive terminal branching of fibers causing enlargement of the motor unit and we have seen this enlargement to be very great indeed in certain cases. It would seem that any treatment which would increase this natural tendency



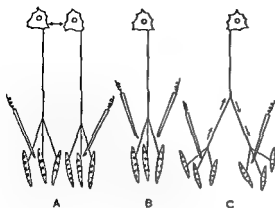


FIG 337 Three types of synchronism of motor unit activity

should be quite beneficial in muscles which are only partially paralyzed

DR RICHARDSON In his paper Professor Buchthal has described the electromyographic changes of muscles in the early stages of acute poliomyelitis and related them to the final degree of paresis. In detail the changes were first a 30 per cent increase in the mean duration of the motor unit action potentials, second an increase in the synchronism of motor unit activity, third an increased incidence of spontaneous fibrillation potentials.

Concerning the increased incidence of spontaneous fibrillation potentials, he found first they occurred in 75 per cent of the poor recovery group compared with 33 per cent of the good recovery group. This is to be expected because the presence of fibrillation potentials is an index of lower motor neuron degeneration. In poliomyelitis the fact that this degeneration arises from irreversible motor cell damage must mean that regeneration of the low motor neuron is permanent. Hence finding fibrillation potentials must be of significance. However the trouble is that the onset of fibrillation in poliomyelitis is as indeed in any other cases of low motor neuron degeneration is delayed for 3 or 4 weeks. Nerve conduction measurements are lost in a matter of hours after the degeneration. For this reason, particularly Brooks in England and most of us now use nerve conduction measurements in the prognosis of treating muscles in poliomyelitis.

Returning to the question of the increased duration of motor unit potentials and synchronism of motor unit activity, Professor Buchthal is going to reopen. I am sure purposely the controversy which has now been waiting for 14 years. This question of synchronism besides the fact that it was first introduced by Clemmesen and Buchthal is a most interesting phenomenon and of course it is not confined to poliomyelitis; it occurs in other lesions.

When they first introduced the term synchronism of motor unit activity, their suggestion was that two motor units beat in unison due to spread of impulses in the damaged cord. This is illustrated in A of Figure 337. In A you see two motor units, two anterior horn cells at the top, and according to Buchthal and Clemmesen there is a spread of impulses across the anterior horn cells with the two beating in unison. Some support for this comes from the fact that one can obtain synchronism in strychnine poisoning and other lesions. However Denny Brown later in 1949 put forth another theory which perhaps has been more generally accepted. This I have illustrated in B with two needle electrodes apparently picking up the same motor unit potential. There is much evidence in support of that as Denny Brown's own work was based on measuring the mechanical response and the electrical response at the same time. Kugelberg and Taverner appeared to complete this study by showing that synchronism could occur on peripheral stimulation of the nerve, therefore virtually excluding the idea that it was due to a spread of impulses in the cord. However as Professor Buchthal has pointed out repeatedly, there is no doubt that at times one can localize two motor potential sources. With this in mind Burns and I suggested the third theory, C, and that was that the residual motor units in poliomyelitis were often branched. Our evidence for that was based on the fact that we sent a stimulus along one needle electrode and got an excellent reflex at about 4 msec. It does seem a most likely explanation of synchronization of motor unit activity as selective destruction of anterior horn cells leaving the large branched units. However Professor Buchthal's suggestion indicates first

that this cannot be served because the residual motor potentials are too great to be accounted for by survival of the large ones and second that measurements of the velocity of the fiber action potential are not compatible with this theory and will I think reopen this whole subject again

Therefore in conclusion whatever the explanation of this mysterious phenomenon of synchronization it is clearly of prognostic importance as is the instance of fibrillation potential. As to its mechanism we need much more study before we can decide

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# *Physical Medicine and Orthopedics in Treatment of Patients with Respiratory Paralysis*

DR JESSIE WRIGHT

In all phases of care of poliomyelitis psychological and emotional factors are of great importance especially in treatment of patients with respiratory paralysis. Anxiety and neuromuscular tension may be obstacles to a sense of ease and confidence which makes breathing easier and more rhythmic no matter what degree of paralysis is affecting respiration. Tension may reflect a feeling of insecurity and besides blocking neuromuscular mechanisms may lead to restriction of blood flow and unnecessary shortening of tissues. All this may contribute to discomfort or to a tendency to deformity as well as poor mechanics in joints and muscles which receive most of the blame for disability. Anxiety and tension also may interfere with co-operation by distracting the patient from a favorable program which has been prescribed. A person does not need to be paralyzed to realize how emotion alters breathing. These factors added to actual paralysis may place respiration at a greater disadvantage than is necessary.

All professional personnel from the onset of illness should show an interest in the patient's personal worth and attainment. This is particularly important in a patient recovering from impaired breathing. Physical treatment or prevention of disability and deformity should not form the central theme of the patient's existence. Educational or pre-vocational guidance should proceed with equal pace as the general condition permits. The physician, the nurse, the medical social worker and the psychologist as well as all other professional personnel can help the patient and family with these problems which may influence the progress toward recovery. The neuropsychiatrist may be one of the consultants for special disturbances. But all those having contact with the patient should be oriented to the multiple needs during convalescence.

Introducing re-education procedures early in

convalescence helps patients to make the transition from necessary ministrations during the acute stage toward greater independence and practical goals. Otherwise patients who have had respiratory distress may become tyrants who resist weaning from mechanical aids.

As soon as the temperature has been normal for 48 hours or when the general condition permits the costovertebral and facet joints should be mobilized and kept free during convalescence. Most often these joints are overlooked while the joints of the extremities are moved in a desirable way from the early days of illness. Freedom of the costovertebral and facet joints is essential to easy migration of the ribs in breathing.

Not only are mobile costovertebral and facet joints essential to optimal breathing but such movement favors proprioception in the joints and muscles of respiration helps blood flow moves metabolic products and limits the discomfort and aching that result from inactivity. Adequate working of the chest bellows helps to blow off carbon dioxide and to take on oxygen necessary to metabolism in all organs and tissues.

When the physician prescribes exercises taking advantage of the momentum of the rocking bed he asks the physical therapist to use an active assisted range of motion for the costovertebral and facet joints of the trunks as well as for the extremities and neck.

When the head of the bed comes up the patient inhales. Then he rests for one or more cycles of rocking repeating the exercise with every second or third excursion of the bed. Next the patient pushes his head backward against the bed chin level at the same time arching the upper back and pulling the shoulder blades together while inhaling as the head of the bed rises. During expiration the physical therapist compresses the lower chest as the head



FIG 338 Applying compress on to the thorax. As the head of the rapidly rocking bed goes down and the physical therapist compresses the lower thorax helping the patient to exhale and moving the costo-vertebral joints.



FIG 339 Releasing compress on to the thorax. As the head of the rapidly rocking bed rises the physical therapist releases the lower ribs helping to mobilize the costo-vertebral joints as the patient inhales.

of the bed goes down helping exhalation (Figs 338 and 339). Both of these maneuvers mobilize the costo-vertebral joints. Next the patient makes active effort as the therapist twists the thorax for mobilization of the costo-vertebral and facet joints. During this maneuver the physical therapist reaches under the thorax with one hand around each side and holds the shoulder blades toward each other to protect the interscapular groups from stretching, as will be seen in Figure 340.

The visceral shift during the two phases of movement of the bed not only moves the diaphragm but also helps to mobilize the costovertebral joints. If the diaphragm is better than the intercostals, further migration of the ribs may be attained by using a rocking bed with chest bellows and shell synchronized with the rocking. This may be seen in the exhibits. The chest shell should be placed high on the trunk to permit as much excursion of the upper thorax as possible.

Position of the head in relation to the thorax will influence breathing. Whether or not the patient is in a tank, lung, or chest respirator, releasing internal positive pressure breathing or on a rapidly rocking bed the effectiveness of any of these aids will be influenced by the rela-

tive position of the head on the neck and the neck on the trunk. The patient's position in bed should be comparable with standing tall with the back of the head high and the chin level which elevates the ribs.

Efficiency of breathing and circulation is affected by the tone of the abdominal muscles. The pull of the tank respirator may stretch weak abdominals. If the chest respirator is not fitted carefully, expansion in the epigastrium may be exaggerated while movement of the ribs may not be attained because the shell has been placed too low on the trunk.

On the rocking bed if the abdominals are weak as the head of the bed rises one sees from a side view that the visceral structures migrate going beyond the desirable downward pull on the diaphragm to cause a protrusion of the lower abdominal wall. In order to encourage a craggy diaphragmatic shift to protect the abdominals from stretching and favor reculation a support should be given to the trunk from over the pelvis as far up as the dependent ribs. The part below the umbilicus should be fastened firmly with graduated support, looser above so as not to limit excursion of the diaphragm. In order to prevent riding upward of the support, a many-tailed scultetus binder may be used carrying the lowest strip on each side under the groin as a perineal strap (Fig. 341).



FIG 340 The physical therapist mobilizes the costo vertebral and facet joints to make breathing easier



FIG 341 A sandbag limits motion of the side of the chest that expands better while the patient concentrates on inhaling motion on the limited side as the head of the rapidly rocking bed rises. A many-tailed scultetus binder holds the lower abdomen to prevent protrusion of a weak abdominal wall as the head of the bed rises

If the diaphragm is good and the intercostals poor it is particularly important to use a support for the lower abdomen or else the upper chest actually may be depressed during expansion in the epigastrium. When the patient is improved enough to have the bed stopped for meals and the back and knee rests are cranked into a semi-sitting position the blood pressure may drop and the patient may feel faint especially if the abdominals are weak. When a transition is made to a more erect sitting position in bed or on the side of it the support of the lower abdomen should be firmer and in slender patients a lower abdominal pad or inflatable mold placed to limit protrusion of the lower abdomen. This also guards against fall in blood pressure which may be enough to cause fainting and further limitation of breathing.

Much of the stiffness seen in spinal joints and extremities can be limited or prevented if the physician seeing the patient early in illness has the interest and courage of his conclusions in limiting the use of tank lungs and in progressing to internal positive pressure breathing carefully regulated or by using the cuirass shell with the rapidly rocking bed. Under the burden of an overwhelming number of patients during seasonal outbreaks or epidemics it may be hard to be at hand to change the prescription for respiratory aids to meet the transitions in the condition of patients. But only in this way will

unnecessary impairment of chest or extremity motion be prevented, circulation facilitated and tolerance developed for subsequent physical and occupational therapy which is directed toward recovery as complete as the original insult to the nervous system permits. Of course tolerance for any of the mentioned activities will depend on maintaining the vital functions peacefully coordinated in this conference by the physiologists, pediatricians and internists who are concerned with such early care which may be lifesaving.

### STEPS IN REHABILITATION

Deformities may be prevented or limited by attention from the first day of illness to physiologic positions of advantage modified temporarily to relieve pain or muscle spasm. Turning the patient in accordance with need and assuring adequate freedom for breathing will add to comfort by helping to regulate limiting pressure on sensitive affected muscles and protecting against passive congestion in dependent parts.

Modified splints and supports especially useful in patients with respiratory paralysis have been seen in the demonstrations at this conference. Careful positioning of body parts including the thorax and spine at appropriate intervals in effect splinting and may be successful in co-operating patients but not usually in young children.

dren many of whom require more formal support of extremities or trunk.

In the event of asymmetric paralysis resulting in expansion of one side of the chest more than the other, a tendency to scoliosis will result. A flat sandbag shaped to the contour of the better half of the chest may be made and anchored so as not to interfere with expansion of the limited side. The weight and size of the sandbag on the good side should be planned according to the degree of inhalation possible on the more involved side so as to avoid too much restriction of total air intake.

Movement of the joints of the spine, thorax and extremities increases kinesthetic sense and makes the patient more aware of the area to which effort is directed. Improvement of circulation gives better distribution of oxygen to all body parts. Such valuable aid is given by the rocking bed besides its aid to breathing. Symptomatic relief is then more easily explained because stasis of blood flow is not likely.

Tolerance for physical treatment or appliances may be influenced by respiratory function as well as by electrolyte balance, nutrition and cardiovascular functional capacity. Transitions from respiratory aids to regular bed to sitting, standing and walking are naturally slower in a

patient with limited capacity for breathing. If one does not appreciate this fact and proceeds to permit fast changes in the program, the patient may regress so that return to mechanical help for this vital function results.

Extremity bracing will follow the same indications as in patients without limited breathing except that lighter weight materials are used to limit exertion. But modification of trunk supports will follow lines already mentioned as giving particular attention to holding the lower abdomen while not restricting diaphragmatic or costal excursion.

Each patient with respiratory paralysis offers a special challenge for formulating a long range plan which will be influenced not only by problems in breathing but also by emotional and personality characteristics by extent of involvement of other muscles of the neck, trunk or extremities as well as by the age, size and physical endowment of the individual. With the Salk vaccine we can look forward to diminishing numbers of new cases of poliomyelitis. More professional personnel should be available for patients with respiratory paralysis so that we can do more efficiently what we know is needed to make the best recovery possible in the shortest time.

## DISCUSSION

DR POLLOCK. In expressing my gratitude for the opportunity which has been given to me to discuss Dr Wright's paper I must at the same time record the pleasure I have had in reading her excellent communication.

The increasing degree to which specialization has overtaken our profession has tended to focus medical attention on progressively narrowing fields of interest so that to many of us the part is now almost more important than the whole. It is both refreshing and invigorating to know that to Dr Wright the patient is indivisible and from the viewpoint of treatment must be considered as a total individual.

She states rightly that the first step in treatment is to establish early in the disease a true doctor/patient relationship since only by so doing can we obtain his active co-operation and if we hope to retain it we must be as honest in our approach as we are realistic in our aims.

In discussing physical medicine and orthopedics in the treatment of patients with respiratory paralysis I need make no apology for referring in particular to the most serious sequel with which the orthopedic surgeon has to deal in the convalescent stage of poliomyelitis—scoliosis. There can be few deformities more crippling to body and soul than the progressive lateral curvature of the spine which may follow a wide spread poliomyelitis.

Every case of severe paralytic poliomyelitis is a potential scoliotic yet many cases show little or no evidence of this complication. Others again with lesions apparently comparable in degree and extent develop a grossly deforming curvature. Can any one of us with confidence name the essential cause? Ghormley suggested with more or less general acceptance that fascial contracture, muscle imbalance and stretch paralysis were the exciting factors in its development. But this is an oversimplification of the complicated interplay of the many factors which give rise to scoliosis and gives little or no guide in the detection of the structure initially at fault and in consequence an indication for its arrest or correction.

In a recent review of 330 cases of poliomye-

litis admitted to the Princess Margaret Rose Hospital Edinburgh during the 10 year period 1947-1957 51 cases showed evidences of respiratory involvement—many of whom required up to 3 months in a respirator.

Of these 51 cases no less than 34 developed a scoliosis—a much higher incidence than that for the group as a whole. It is not without significance that in this group alone i.e. that showing respiratory involvement a scoliosis was discovered clinically in 12 cases before the child was allowed out of bed and therefore before gravity could play a role in its production.

It seems reasonable to assume that weakness of the long spinal muscles the sacrospinalis the quadratus lumborum trapezius and iliopsoas etc. of one side—like the slackening of the guy ropes of a tent—will result in a spinal tilt to the opposite side or the reverse will occur if there is contraction. In one case progress may go on to deformity and in another the condition remains unchanged.

Examination of 21 cases by fluoroscope suggests that the weight which may tip the balance in favor of a progressive scoliosis is paralysis of one leaf of the diaphragm with a segmental or more widespread weakness of the intercostals of the same side occasionally aggravated further by involvement of neck and lateral abdominal muscles and at a later period by the effects of gravity and leg inequality in length and muscle power.

In many of these cases the application early in the postfebrile period of the principles of treatment suggested by Dr Wright may limit if it does not completely arrest the tendency to increasing deformity.

We know from experience that the acme of functional recovery following poliomyelitis can be obtained only where in addition to recovery of muscle power there is present a normal range of joint movement. It is in the light of this fact that Dr Wright's insistence upon maintaining the costovertebral joints mobile must be viewed. Also Cobb has shown that it is as important to mobilize the joints of the spine as it is to keep the articulations of the extremities supple.



FIG 342 Gross lateral scoliosis with erosion of the ribs. In this case the convexity was so gross that erosion of the ribs has taken place as a result of pressure.



FIG 343 Scoliosis showing spinal deformity.



FIG 344 Deformity of rib which accompanies a severe scoliosis. Note the extreme degree of obliquity of the ribs.

Therefore it is only logical that we should apply to the costovertebral and facet joints those methods of treatment advocated by Dr Wright and already in common use where the joints of the limbs are affected.

Figures 342 and 343 show the extreme degree of spinal deformity. The first one looks like a kyphoscoliosis but is in fact a gross lateral scoliosis with almost erosion of the ribs. Roaf has recently pointed out that the axis of vertebral rotation in the dorsal spine corresponds to a point just anterior to the posterior margin of the body. We know that this corresponds to the nucleus pulposus and that under normal conditions a line drawn through the nucleus will lie just anterior to the heads of the ribs. Normally these exercise a balanced pressure on the body and transverse processes. Roaf claims that in a scoliosis the position of the rib relative to the axis of rotation of the vertebral body changes so that the ribs on the convex side tend to fall behind the axis of rotation whereas those on the concave side lie in front of it. Figure 344 seems to suggest that this is so but in addition it does

demonstrate the degree of deformity of the rib which accompanies such a severe scoliosis—a point of considerable importance for not only do the ribs lie in extreme obliquity but they become hooked at the neck in such a way that any pressure applied to the convexity of the rib in an attempt to lessen the rib hump will cause direct pressure upon the transverse process and thus may lead to an increase in rotational deformity rather than to its diminution or correction. I think that in the figures particularly on the convexity you can see the hooking of the remnants of the ribs and how they may press



on the facets. Therefore in the process of mobilization of the costovertebral joints it is essential that pressure be applied correctly if Dr Wright's objectives are to be achieved. Perhaps the fiercest spur to intensive and early physical therapy is the late results of surgery. An operation to fuse the spine all too frequently is the final dramatic act in an attempt to arrest a progressive deformity which has deteriorated in spite of every other form of therapy. Unfortunately spinal fusion is not as successful a solution to our difficulties as we would like to believe. In a recent review of over 600 cases of scoliosis in which 58 cases (or 7%) were successfully subjected to a spinal fusion operation Winchester found that only 14 per cent showed no further relapse. That failure to fuse the spine over a sufficiently extensive area may have been responsible for some of the relapses is possible but the greater number were due almost certainly to two factors frequently overlooked: (a) in attempting to produce a spinal fusion we are apt to forget that we are dealing with a growing structure subject to Wolff's laws of stress and strain and (b) that the initial causative factors are still acting. Therefore it is not altogether surprising that so long as the original etiologic factors continue to exercise their deforming influence the growing spine will still respond and some degree of relapse is consequently almost inevitable. A more critical physiatric analysis of the essential etiologic factors may lead to the future use of myotomy or neurectomy of the contracted or overacting muscles with arrest of the deforming process and a better end result than that obtained at present by surgery.

At the moment our sheet anchor in the prevention and treatment of those deformities which follow paralytic poliomyelitis with respiratory involvement is the early and wise use of those physiotherapeutic methods so admirably described by Dr Wright.

By the adoption of Dr Wright's method of treatment fascial contractures are stretched, muscle tone is increased and pulmonary function improved.

Increased vital capacity with improved oxygenation of the blood and better cardiac function must react favorably on muscular function generally and so lessen the tendency to spinal

curvature and rotation and thus establish a salutary cycle of improvement in place of that vicious one which leads on to deformity.

Dr RAE Dr Wright has emphasized that all professional personnel concerned with the treatment of respirator patients should be oriented to the multiple needs of these patients during convalescence. The full co-operation of medical and ancillary personnel is required to bring these patients to maximal function and independence commensurate with their limitations. The ultimate goal is to return the patient to as normal an environment as possible. In many cases severely involved patients have been able to return to productive employment. Social service workers and vocational counselors contribute significantly to the restoration program.

I agree with Doctor Wright that physical treatment should not be the entire theme of the patient's program. Appropriate counselling, guidance and planning in psychological, social and vocational areas should proceed at an appropriate pace. There needs to be a correlation between such planning and the physical treatment program.

There is little doubt that physical treatment plays an important role in the treatment of the after-effects of poliomyelitis and the functional restoration of the patient. A program of physical care designed to promote a maximum of functional restoration must include measures to prevent and minimize deformities and mobilize the patient to as high a level of efficiency in body mechanics as possible to co-ordinate and strengthen neuromuscular units in which function remains and to make effective use of assistive devices, orthopedic apparatus and surgical procedures.

Although Dr Wright was unable in the time allotted to give a detailed description of the physical treatment of poliomyelitis she presented some interesting ideas regarding mobilization of the joints of the spine and the thorax. She has pointed out that these joints are often overlooked in the mobilization program and that freedom of motion in these joints is necessary for optimum breathing. One technique that seems to be effective in mobilization of the chest and that was not mentioned in this paper is glossopharyngeal breathing. Glossopharyngeal

breathing is a technic for ventilating the lungs that does not require the use of the muscles of respiration. The muscles of the mouth and pharynx are utilized to force air into the lungs under positive pressure. This type of breathing enables patients with reduced vital capacity to inhale a far greater amount of air than is possible with any of the deep-breathing machines. Some studies we have carried out seem to indicate that a more compliant chest can be obtained by periodically expanding the thorax and lungs with deep glossopharyngeal breaths. The work of breathing is reduced with a more elastic chest. In some patients this may mean more time free from respiratory aids or in some cases it may permit the use of a less confining respiratory aid.

One of the most distressing features of poliomyelitis is the tendency to develop deformity for the disease is one of tissue contracture as well as of muscle weakness. If spasm which occurs so frequently in the acute phase of the disease is allowed to persist permanent shortening of muscular and ligamentous structures may result. Appropriate physical measures can minimize or prevent such tissue contractures. The primary cause of deformity is persistent faulty position which may be the result of untreated muscle spasm, muscle imbalance, forces of gravity or poor body mechanics.

In the acute phase of poliomyelitis adequate rest of course is essential but this does not mean that the patient should be immobilized completely. Physical treatment should be started as early as possible and may consist merely of

the use of heat often in the form of full prone packs and gentle passive motion to keep the patient mobilized.

It is not always desirable to obtain a completely normal range of motion in all joints but merely to provide adequate mobility for functional purposes. Many of the respirator patients with severe involvement of all four extremities and trunk will never be able to walk again and therefore need only sufficient range of motion to permit them to sit comfortably and to be dressed easily. In fact in some patients it may be advantageous to leave some tightness to support severely weakened muscles. Mild to moderate symmetrical tightness of the back might be used to stabilize the trunk when sitting. Mild residual shortening of the muscles about a flail shoulder might minimize separation of the glenohumeral joint that may occur when the patient is upright and the arms are in a dependent position. Normal alignment of body segments usually can be preserved by maintaining or restoring muscle and joint mobility by proper positioning of the body and by using apparatus to support weakness.

Various types of orthotic devices and braces may be of great value in (1) prevention of deformity (2) support of weakness (3) assistance in the performance of useful activities. Orthopedic devices and braces should not be employed as merely a last resort but used intelligently to achieve any of the above objectives.

I would thank Dr. Wright for an interesting paper and for bringing to our attention certain concepts and new techniques for mobilization of thoracic structures.

# Treatment of Early Paralytic Scoliosis

DR ROBERT BENNETT

Early recognition and treatment of paralytic scoliosis is necessary to prevent the development of significant structural distortion of the spine thorax and pelvis. Not all of the factors that cause persistent lateral and rotary deviations of the spine can be prevented or corrected but the development of serious structural changes can be controlled in most cases by adequate care. Preventing, correcting or minimizing scoliosis depends on (1) very early recognition of persistent lateral and rotary deviations of the spine (2) recognition of all factors that could possibly bring about persistent faulty alignment of the spine (3) constant attention by the physician (4) full co-operation of the patient and his family.

X ray visualization of the spine is necessary to detect the earliest deviations from normal alignment. Such roentgenograms should be taken in the erect sitting or standing position. Roentgenograms taken in the supine position are of little value because they minimize the effect of weight and the attempts to stabilize the spine by weakened musculature or poorly balanced muscle strength. Because roentgenograms should be taken early in the course of paralytic disease sitting roentgenograms are preferable to standing views. Patients with severe paralytic disease can be brought to a sitting position safely long before they can be permitted to stand. When spinal deviations exist particularly in the growing child roentgenograms should be repeated every 3 or 4 months to determine the response to treatment and the effect of increasing age, weight and activity. It is always wise to x ray the spine both with and without supportive and corrective apparatus to be sure that such apparatus is fulfilling the purpose for which it was applied.

By clinical and x ray visualization early paralytic scoliosis may be expected to follow one of four patterns or types. Occasionally in the very early stages several types may occur simultane-

ously. Combined types are common and expected in the later stages of the development of severe structural scolioses. An understanding of the basic types makes evaluation and treatment of the combined types less difficult and far more specific. An understanding of the pathogenesis and significance of the individual types enables us to analyze the various combinations that may occur.

**Type 1** This type is characterized by (1) a lateral tilting or shifting of lumbar 4 on lumbar 5 on sacral 1 (2) a compensatory curve in the mid or high lumbar region (3) a level pelvis when patient is sitting or standing. There are five important causes of type 1. These causes may act singly or in multiple combinations: (1) pre-existing developmental faults in the lumbosacral region (2) weakness or imbalance of intrinsic spinal muscles (3) weakness or imbalance of quadratus lumborum and/or iliopsoas (4) asymmetrical contracture of the iliobulbar band which may occur on either side of the convexity of the curve (5) asymmetrical loss of bulk at the hip and thigh. This type is significant because faulty lateral take-off at the base of the spine puts a strain on the entire spinal column and tends to exaggerate any weakness and imbalance that might influence the alignment of the spine above the lumbosacral region. The faulty lateral take-off may accelerate a curve existing above or conversely may act as an anchor to control a curve above. Further deviation in the lumbosacral region with increase in the lumbar curve may be expected (Fig 345).

If the faulty lateral take-off in the lumbosacral region cannot be corrected one of three patterns of progression may be expected.

1 The ilium on the side of the acute lumbosacral angle may be raised and the spine above regain normal alignment.

2 The lateral take-off will increase and the lumbar curve secondary to this deviation will progress rapidly. The dorsal spine will remain in good alignment except for its lower portion.



FIG 345 Type 1 scoliosis Early (left) and common pattern of progression (right)



FIG 346 One method of stretching the left lumbosacral angle without danger to the left lumbar curve

which has become the base of the lumbar curve. The rib cage will not be significantly distorted.

3 A compensatory curve will develop in the dorsal region to balance the lumbar curve. This dorsal curve may progress out of control, cause severe distortion of the thorax and become the major problem. In this pattern the lumbar curve usually decreases as the dorsal curve progresses.

Treatment of a type 1 pattern should emphasize the following 6 points:

1 Every attempt should be made to mobilize immediately the acute lumbosacral angle. This may be done by passive or active stretching. X-ray visualization of the lumbosacral region must be available not only to determine the extent of deviation and its response to treatment but also to determine if a structural fault exists that would limit the possibility of realignment of the spine with treatment (Fig. 346).

2 If asymmetrical contracture of the iliotibial fascia exists, it must be released immediately either by conservative stretching or fasciotomy. Conservative stretching obviously should be attempted first, but if there is not a very definite release of contracture in a reasonably short period of time, surgery must be carried out. In the early stages of the development of a type 1 asymmetrical tightness of the iliotibial fascia is equally important on either side. If it exists on the side of the deviation, it will pull the pelvis down, increasing the lumbar curve. If it is

present on the side opposite the lateral deviation, it may stretch out the lumbosacral region and thus increase the lateral deviation.

3 A gluteal pad under the side of the acute lumbosacral angle should never be used without very serious consideration and X-ray study. It is always a temptation to realign the lumbar curve secondary to the lumbosacral deviation by lifting the pelvis on the side of the convexity of this lumbar curve. Unfortunately, this usually results in an increase of the lumbosacral angle.

4 As stated previously, one of the greatest dangers of this type of early deviation is the later development of a compensatory shift of the thorax to the side opposite the lumbosacral deviation. This shift of the thorax takes place rapidly in growing children, particularly after 9 years of age. In a pure type 1 deviation, uncomplicated by severe trunk weakness, this is by far its most important aspect and must be counteracted by support and stretching.

5 A true type 1 pattern of deviation cannot be controlled or significantly altered by any type of back brace or corset. There is no way through spinal support to obtain pressure low enough in the lumbar region to affect the lumbosacral angle. Spinal traction is of some limited value and should be attempted. This can be accomplished by repeated hanging from an overhead bar, use of Sayre head sling attachment of a head traction to a corset or back brace. However, traction may prevent the later shift of the thorax and certainly should be used when there is any indication that this shift is taking place. A firm, well-fitting corset with its top shifted off against the shifting of the thorax should be applied immediately if such thoracic shift is noted.



FIG 347 (Left) Type 2 scoliosis (static) (left) with gluteal pad under right hip and thigh (right)



FIG 348 (Right) Type 2 scoliosis (dynamic) Early (left) and common pattern of progression (right)



FIG 349 Type 3 scoliosis Early (left) and common pattern of progression (right)

6 Any activity that causes downward pressure against the lumbosacral region should be sharply limited. This means that there should be marked restriction of both sitting and standing periods. The patient should be kept in a reclining or semisitting position as much as practical and consistent with the care of other skeletal problems.

**Type 2** This pattern of early spinal deviation is characterized by (1) a pelvic tilt downward on the side of a midlumbar convexity and (2) a normal lumbosacral take-off. This type must be recognized as having two forms—the static and the dynamic. The static form is common. It is simple to correct and important only as it influ-

ences other types of deviations. The dynamic form is fortunately not common. It is almost impossible to correct. It usually results in severe structural changes in the spine and in overall functional handicap.

We will discuss the static type first. Figure 347 shows stretching of the lumbosacral region and was a part of the discussion of type 1. This is simply one method for stretching the lumbosacral region. This is a typical static type 2, the common causes of which are:

- 1 Asymmetrical loss of bulk in the hip and thigh causing the pelvis to tilt when the patient is sitting.

- 2 Discrepancy in lower extremity length tilting the pelvis when the patient is standing.

- 3 Faulty fitting of lower extremity apparatus so that the patient tends to sit down in his brace. The brace may be too short above the knee or the upper thigh band may be too deep or the knee pad too loose. Tilting of the pelvis for these reasons causes a simple functional curve that has little importance in itself. However, it may act as an accelerating factor in the progression of other curves.

I think the treatment of type 2 is obvious so we will go on to the dynamic which is far more important.

**Dynamic Type 2** This form is caused by severe imbalance of strength of trunk musculature of hemiplegic pattern of imbalance. This

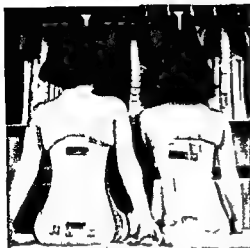


FIG 350 (Left) Straight corset (left) and with top shifted to left (right)



FIG 351 (Right) Pöntenogram of patient with and without shifted corset

imbalance is almost invariably coupled with iliotal fascial contracture on the side of the weakened musculature. The pelvis is kept in a persistently tilted position by the unopposed use of lateral trunk musculature lifting the patient on the side of the strength and the tight iliotal fascia depressing the pelvis on the opposite side. Figure 348 shows the progression of such a curve. The treatment of this dynamic pattern is extremely difficult.

Certainly the iliotal fascia on the low side if tight or snug should be stretched or its mobility periodically determined and then sectioned by surgery if it cannot be otherwise mobilized. In this type we believe that a corset with head traction should be utilized for its pressure against the pelvis in the attempt to help the imbalance.

**Type 3** This is a common type characterized by a long C curve. It may occur in any level. Figure 349 shows a simple one of the dorsal lumbar region and its usual form of progression. It shows a curve slightly higher still of type 3 and on the right its common progression. It is characterized by having a normal take-off in the lumbosacral region and a fairly level pelvis commonly combined with a type 1 deviation and if such a combination exists a severe single or even a double curve may be present very early.



FIG 352 Type 4 scoliosis Early (left) and common pattern of progression (right)

The treatment is to obtain and maintain symmetrical mobility of the spine and is the key to the control of this deviation. This does not mean that normal mobility is desirable in each case. If muscle weakness or imbalance exists symmetrical tightness may be necessary to maintain alignment. In general it can be stated that mobility must bear a direct relationship to underlying strength.

Many different types of spinal support have been designed for this type of curve; each has advantages as well as disadvantages. We use a basic Hoke type corset for spinal support as we feel that it can be made to fit well and can be

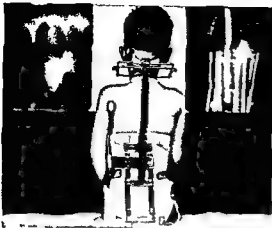


FIG 353 (Left) Adjustable head traction attached to corset (back view)



FIG 354 (Right) Adjustable head traction attached to corset (side view)

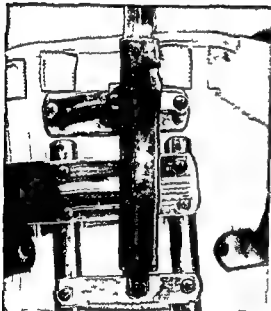


FIG 355 Traction bar may be adjusted both vertically and horizontally

reinforced. Also it can be shifted at the top to the right or left to combat the tendency of a right or left dorsal lumbar curve of this type. Figure 350 shows a simple corset with its top shifted.

Figure 351 shows roentgenograms of a patient with a left dorsal lumbar curve and a corset with its top shifted to the right showing the influence of the underlying spine.

Type 4. This pattern is characterized by an

angular curve at the level of dorsal 4, 5 or 6 and the existence of a long moderate dorso-lumbar curve with minimal rotation. Usually there is a good take-off in the lumbosacral region and the pelvis is fairly level. The causes of this type of curve are not completely understood. Certainly weakness or imbalance of the intrinsic spinal musculature of the upper dorsal region must be at fault. In many of these patients we find rather severe loss of strength in the muscles of inspiration and very frequently we find imbalance about the shoulder girdle. This curve is of great significance because it tends to develop the severe structural curve of great cosmetic as well as physiologic importance. Figure 357 shows the typical pattern of progression.

As previously noted, early recognition is the most important point in treatment of type 4. Certainly conservative methods of care are in capable of correcting significant structural scoliosis. Prevention of significant structural scoliosis is possible but can be achieved only through early recognition and control of all those factors present in the individual patient that might cause persistent faulty alignment of the spine. General treatment will consist of (1) limited activity, (2) corrective mobilization and muscle strengthening, (3) spinal support and (4) control of bodily weight (Figs 353, 354 and 355).

Treatment is tedious and requires the constant attention of the physician and the full co-operation of the patient and his family. A knowledge of the pathogenesis of the four common types of early deviation of the spine is of great help

in the prescription of care for these individual types as well as in determining the important features and the care of combined types

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of our advice to the contrary to cope with the child's natural tendencies toward activities and widely unrestricted movements

Duly appreciating the endeavors of my colleagues and particularly of Dr Bennett to look for conservative measures in preventing severe paralytic scoliosis and trying myself to do the utmost in the same direction I must confess that at least with little children and under difficult social and economic conditions the outlook for scoliosis originating from paresis or paralysis of intrinsic structures of the back and treated by conservative methods is not favorable

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- 12 Grossiord A Ciosi Frenay C Held J P and Beaupere G A propos de la prevention des scolioses poliomyelitiques in Proc Second Internat Congr Phys Med p 491 Copenhagen 1956

DR GROSSIORD I congratulate the speaker on his very interesting report nearly every line of which is worth meditating On the whole I am in full accord with the remarks made on the four principal types described by Dr Bennett

Our topic is a highly specialized one as is that of the paper and the exhibit my co-workers and I have prepared on the subject the consideration of the possibilities for prophylactic treatment of poliomyelitic scoliosis that is to say resolute consideration of the purely medical aspects of the problem when confronted with a case of poliomyelitis of recent onset

Our experience is now based upon 9 years of work in our Centre de Rééducation de Garches (Garches Rehabilitation Center) and more than 7000 cases of paralytic poliomyelitis most of them severe all having been hospitalized at some time and many of whom presented spinal complications For the past several years all cases of poliomyelitic spine have been subjected to minute investigation and given standardized clinical x ray examinations

If we attempt to summarize in a few words the pathogenesis of poliomyelitic scoliosis which is in reality extremely complicated we find four essential factors

1 The muscular imbalances created by the disease which produces this condition in perfection and it seems to us beyond question that the involvement of the spinal muscles themselves is by far the most important in this connection the danger arising from other possible factors of imbalance seems to be limited Actually we must first agree on what is to be called normal spinal musculature! Slight involvements discoverable only by delicate diagnostic procedures must not be overlooked

2 Aponeurotic fibromuscular contractures and here again directly paravertebral contractures are of greatest importance

## 3 Aggravating effect of "mise en charge

4 Disorders no less important of growth according to the old Law of Delpech

Granting that although everything will be done to perfect attention and nursing in the acute stage and to see the patient through the first or second month in the best possible condition we are nevertheless bound to admit a number of points

1 A muscular imbalance if it is definite never will be completely compensated the antagonistic muscle pair regaining strength but remaining imbalanced

2 When a well-defined state of contracture exists in the paravertebral structures it is difficult to reduce and if that can be achieved to maintain the results obtained "postures dorsal or decubital frames corsets etc have only limited effect

3 Apparently we can do nothing against the growth itself of the spine at least by simple measures

4 Thus there remains to us in the last resort one factor above all upon which we can act when there is danger the management about mise en charge we can in fact control it in some extent.

## SELECTION OF CASES

The danger of scoliosis in paralytic poliomyelitis is such that it is essential to be able to say with relative precision in a given case whether or not the spinal column is in danger

This evaluation scarcely can be made in the first weeks

In general it becomes possible only at the end of the first month sometimes a little later and we believe that strict decubitus should be maintained so long as one has not been able to form an opinion. What this actually means is that the sitting position is ruled out during the first month of the disease. This spinal balance sheet will be

1 Clinical based on detailed testing of the trunk muscles above all those of the spine

2 Radiologic based on three standard frontal plates

(a) corrected decubitus

(b and c) forced lateral inclinations right and then left in the same position

We may certainly agree with the speaker that a plate of the lying position minimizes the danger which a plate of the sitting position objectifies but it may be objected in this that a plate of the sitting position is subject to many

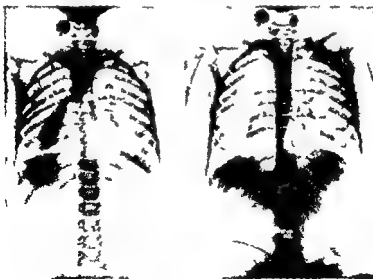


FIG 356 Aspects of rotation reduced by use of a pillow under the atrophic buttock

causes of error certain of which are detailed in our exhibit and that these causes of error are such that the radiologic evidence will lack the comparative value needed in order to observe the development properly. A corrected plate of the lying position presupposes correction of pelvic imbalances from atrophy of one buttock (Fig 356) lowering of one half of the pelvis by retraction of the tensor (Fig 357) if necessary a cushion is placed under the atrophied buttock or one thigh is flexed while verifying the compensation on the iliac spines by using a spirit level. The pelvis being perfectly level any rotation (Fig 359) or inflexion becomes significant and can be calculated.

To this plate of corrected decubitus for the first radiologic balance sheet we systematically add two more decubital roentgenograms these in forced lateral inflexion the procedure being supervised by the physician. Any noteworthy segmental asymmetry in extensibility should be considered as a serious sign of incipient scoliosis (Fig 358).

Thus we believe that we are able to differentiate with this clinical x-ray balance sheet.

1 Cases in which the danger appears to be absent or virtually absent the trunk musculature has been spared and the spine is completely

normal the spinal column is well aligned and its lateral inflexions quite symmetrical the prognosis is very favorable in all likelihood no scoliosis will appear later even if the child has one leg shorter than the other. We will not say that the possibility of scoliosis appearing is nonexistent caution always is indicated and the spinal column always should be kept under close observation after poliomyelitis. We shall say only—but this is on the basis of considerable follow up—that whenever we have based such a prognosis upon wholly favorable elements the facts have never failed to bear us out. Perhaps they may so fail tomorrow but we do not believe they will.

2 Cases in which there is danger the gravest doubts must be entertained and no attempt should be made to rush through the stages of re-education. We remarked above that limiting the mis-en-charge is one of the surest means of reducing the danger.

The various aspects of the treatment certainly should be accompanied by all necessary rehabilitative measures hot water balneotherapy analgesic exercises to strengthen weak muscles measures to combat contractures closely fitting plaster casts at night with corrective hyperextension when possible.

Re-education in standing and walking posi-

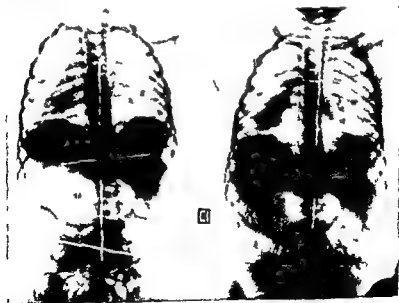


FIG 357 Scoliosis largely reduced by the correction of the pelvic imbalance (due to the retraction of the tensor of the fascia lata)

FIG 358 The aspect of a corrected prone decubitus is not normal. The reflexions are asymmetrical. The patient was lost to follow-up. Subsequent development of marked scoliosis.



tion which is infinitely less harmful than the sitting posture undoubtedly should be begun quite early.

Certainly erythra should be done to ensure that the child's essential physiologic needs are met in spite of the treatment.

But we are firmly convinced that in this effort to reduce the danger of scoliosis when one knows it to be present in a case of poliomyelitic spine postural restrictions as to permission for the standing posture and also all the sitting, position play an important part for 6, 12 or 18 months and sometimes longer.

An infinite number of types of cases is possible in practice.

(a) the most favorable in which the spinal muscles begin to recover their strength very quickly and the spinal column remains perfectly aligned—cases in which great liberty of movement will be allowed quickly while keeping the spinal column under very close observation by means of corrected decubital plates every 3 to 5 months.

(b) the most severe which will be kept lying down most of the day for a long time except for periods of ambulatory re-education.

(c) those cases in which one will permit 2 hours standing posture daily for example but no sitting or complete freedom to stand but



not sitting or freedom to stand during the morning and to sit at meals, etc.

The means for carrying out the essential measures is afforded by the character of plate in all its possible variations included or not which the child takes with him on vacations with his family which he uses in going to school and on which he plays.

It is certainly not simple to gain acceptance for it; the parents must be convinced which is not at all impossible and experience shows that the results pay off. But that we pretend to eliminate the danger of scoliosis by these restrictions for growth continues even when the child is lying down but we believe that it would be infinitely less harmful to the spine of a child lying down 70 or 72 hours out of 24 than in a

child permitted to sit even though he be well supported by a corset and *a fortiori* if he is not—which you will agree is common enough

However in practice we encounter a major difficulty connected with age

If it is a question of a very young child 1 or 2 years old whose condition is such as to por-

tend the later development of scoliosis purely medical means would be of little avail one could limit the risks to be sure but with 10 12 or 14 years of growth ahead how can one insure proper precautions? The solution is to be found only in repeated orthopedic correction by means of special plaster casts or in surgical

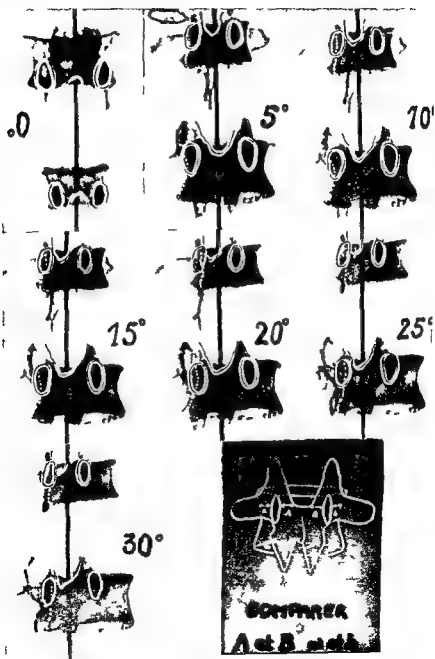


FIG 359 Table of rotations

measures. But what is one to do in the case of the very young?

However if we are faced with a child of 11 or 12 for whom the dangerous years of growth are now limited we believe that the danger is great enough so that we can strongly recommend the restrictions of the application of mise en charge of which we have spoken. These will be more or less long lasting and more or less stringent but we have come to admit the fact that in certain difficult cases in which nevertheless victory appears probable these measures are of value for several years. We have followed and are still following numerous patients who have undergone such measures. Let no one object with generalized psychological arguments stressing how painful it is thus to keep a child fix on his back for 2 or 3 years! First of all walking it is not strict at all many children in that position we fear the result of the restrictions are often much better accepted than one would expect and that the children suffer relatively little from them. Finally the result of bringing these children to age 15 with a straight spine justifies all these efforts both from the physical and psychological point of view.

Let us make it clear that we had no intention of addressing ourselves in this brief paper to the problem of treating cases in which scoliosis of some magnitude is already established. This problem is one for full scale orthopedics. What is of interest to us within the scope of our work is preventive treatment such as

- 1 Preventive treatment in which the constant care in the acute phase is unquestionably the best possible rapid alleviation of pain and spasm.
- 2 Preventive treatment in which selection of posture requisite of spinal column in danger is a prerequisite of the selection necessarily being based on fullest clinical x ray semeiology.
- 3 Preventive treatment which in our view must in every suspect case be based essentially on a systematic and cautious approach with regard to the application of mise en charge.

We shall give no figures as to the length of treatment for they depend on the circumstances of each individual case but it seems quite clear that such an approach permits



FIG. 360. Lift thoracic gibbus facing a spinal column normal at that level but presenting a discrete scoliosis at a lower level.

- (a) the closest supervision of the spinal equilibrium over a longer period of time
- (b) strengthening of the trunk muscles and consequent reduction of the harmful effects of the first liberation of mise en charge and
- (c) gaining ground on the danger period a gain which will to be sure be negligible at those 12 or 13 years old.

Actually in this paper we have considered nothing beyond ordinary cases our clinical material not extending to severe chronic respiratory cases however we have frequently encountered

the extremely delicate problem of possible relations between progressive costal gibbosity (in which the ribs tend toward the vertical) and a dorsal spine which sometimes seems definitely to turn following the movement but which may also retain its normal appearance (Fig. 360). Must not failure of the muscles originating at

the apex of one hemithorax—scalenus and primary intercostals—be held responsible in certain of these cases? At any rate it is a fact that treatment of these cases is particularly difficult and when there is a dorsal scoliosis evolving rapidly along with a gibbosity of this kind postural restrictions will not modify it.

# *Surgical Methods of Improving Scoliosis in Poliomyelitis Patients*

DR CARLOS OTTOLENGHI

This is a critical analysis of the surgical methods employed for the improvement of poliomyelitic scoliosis. It will not be a statistical paper as that already has been provided most competently by Cobb in the Second International Poliomyelitis Conference in 1951 in Copenhagen.

In our files we have records of 1634 cases of scoliosis of different etiologies. Before we enter into the surgical treatment we have to make some general remarks as follows:

1 In paralytic scoliosis there is a muscular disequilibrium which we do not find in idiopathic scoliosis.

2 The earlier paralytic scoliosis appears after the attack the more severe is its evolution.

3 Poliomyelitic scoliosis does not appear or is mild in subjects whose growth was complete before paralysis occurred.

4 The growth factor is fundamental for the prognosis, evolution and treatment of poliomyelitic scoliosis.

5 It is very important to know the evolutionary prognosis of the curves. This has been studied by Ponseti and Friedman and by James. James points out that in cases of thoracic scoliosis commencing between the ages of 0 to 3 years at the age of 10 the curves are above 70°. When scoliosis appears between the ages of 5 and 7 at 10 years of age 80 per cent of the cases have a curve of 70° and when scoliosis

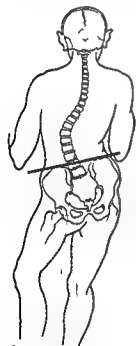


FIG 361 Upper oblique pelvis primitive scoliosis

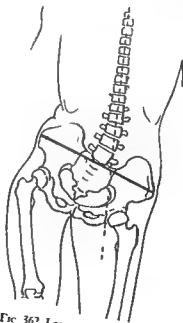


FIG 362 Lower oblique pelvis secondary scoliosis



appears at the age of 10 65 per cent at the end of the growth period have a curve of about 70°. This means that we have a good basis to establish the prognosis.

The objectives of surgical treatment are as follows:

- 1 To correct progressive deformation
- 2 To avoid instability
- 3 To diminish fatigue and pain features generally present in older children or adult cases

Associated paralysis could be:

- 1 Scoliosis without paralysis of the upper or lower limbs
- 2 Scoliosis with paralysis of the upper or lower limbs

In our experience we have found that the paralysis of the scapular muscles does not affect or affects in a very small degree the appearance of scoliosis contrary to what was maintained by Colonna and Vom Saal.

We have scoliosis with paralysis of the lower limbs which may be peripheral in which we find a shortening of the limbs and where very rarely do we find structural scoliosis with the angulation and rotation that characterizes true

scoliosis. Generally they are scoliotic attitudes. Oblique pelvic scoliosis is of two types the upper and the lower.

Figure 361 shows one type of oblique pelvis. This would be the type of oblique pelvis with high variety primitive intrinsic scoliosis.

Figure 362 shows the second type of oblique pelvis the low type scoliosis being secondary to deformation of the pelvis. In these cases when scoliosis is secondary to deformation of the pelvis and when it is straightened scoliosis disappears (Fig. 363). The curve is mobile and secondary to pelvic deformation. These cases as Dr Bennett has pointed out must be studied following Irwin's principles.

But the other type the high variety requires attention because if scoliosis is primary and rigid when the pelvis is corrected scoliosis becomes worse (Fig. 364).

We shall now review other points that are of interest for the indication of surgery and they are as follows:

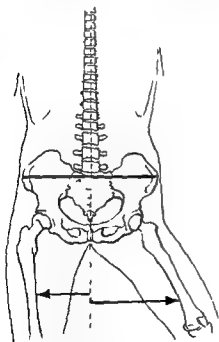


FIG 363 Correcting deformity in the pelvis makes the secondary scoliosis disappear

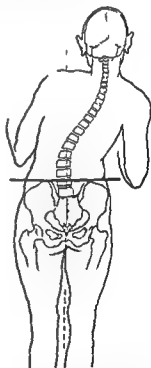


FIG 364 Scoliosis becomes worse when the pelvis is corrected if the scoliosis is primary and rigid

1 The longer the patient rests in a supine position during the growing period when suffering from paralysis of trunk and abdominal muscles the better will be the evolution of the scoliosis

2 In some cases in spite of being immobilized in bed deformation sets in. This is possibly due to fascial contractures

3 Alternating gait is a serious factor tending toward a worsening of the scoliosis

4 Gravity in the absence of an efficient support worsens static conditions of the weakened spine

5 In patients who use crutches because of weakness of the lower limbs the curve has less tendency to increase

We must consider the over all aspect of scoliosis in these cases not only with regard to the spine in these cases particularly in the paraplegic cases we must try to see whether or not an improvement of the scoliotic curve also can condition an improvement of health in general. This we must always take into account

We shall now review the different surgical methods

The diagnosis of scoliotic curves is easily done in cases of poliomyelitis because if we place the patient in the position shown in Figure 365 the primary curve is the one we find more easily

Here you see some points that we consider of interest and importance

Correction of the curve must be done before the operation. We understand that this is important and in this manner we can determine the number of vertebrae that must be corrected and the degree of flexibility of the spine. Angulation and rotation is corrected by means of the Risser type corset following the different techniques of this author. The primary curve must be fixed if possible one or two vertebrae above or below the end

The operation can be done when correction prior to the operation exceeds 25 or 30 degrees if the curve in scoliosis is over 50 degrees. If the secondary curve is not entirely corrected the primitive curve must be straightened or corrected only to that point where the secondary curve compensates it otherwise there will be an aggravation of the curve

Here is a routine case of a long curve 60 degrees (Fig. 366). Once corrected we see it is straightened (Fig. 367). Here it is in a plastic cast with a window and here are the marks (Fig. 368) where we are to practice this operation. This was the case of a young girl of 8

If the curves are flexible evidently in correcting the primitive curve we correct the secondary one but it on the other hand as we said these



FIG. 365 Body positions for making diagnosis of scoliotic curves

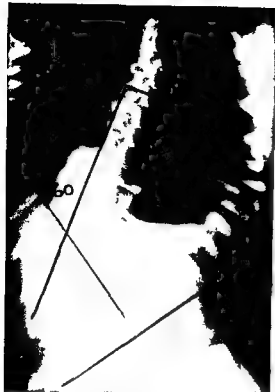


FIG 366 (Left) Routine case of a long curve of 60

FIG 367 (Right) Long curve of 60 after correction with plastic case (with window)

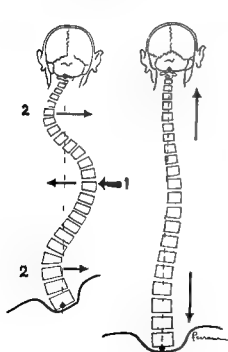


FIG 368 Diagram illustrating correction of primary and secondary curves

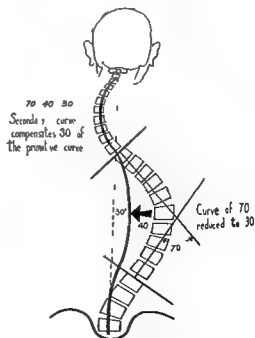


FIG 369 Diagram illustrating how the secondary curve is only corrected to 30 if the primary curve is corrected to 30°



FIG 370 Patient in plaster cast showing how the plaster is open sideways and fastened with adhesive tape (3 positions)

curves are not flexible and if for instance this curve of 70 degrees is corrected only to 30 degrees the secondary curve also will have to be corrected to 30 degrees (Fig 369) to compensate or otherwise we would have a decompensation of the curve. Naturally this is done on the basis of roentgenograms taken routinely.

Some points concerning the technique are worthy of mention and they are the following:

We have to be very careful in the preoperative period to make a respiratory and circulatory test. Then during anesthesia and immobility we must be very careful. We suggest that the operation should be done through a window but we open the plaster cast sideways because we have lost one patient 12 years old because we were not in time to open the plaster in order to practice thoracotomy.

Figure 370 left shows a patient in bed and you see that we have the head up. Here you see how the plaster is open sideways and how it is kept closed with adhesive tape because if anything goes wrong it is easy to open it. Figure 370 middle and right illustrates the same thing.

With regard to technique (Fig 371) during the growth period fusion must be done on the convex side when growth is ended on the concave side.

In Figure 371 left and right we have the same examples as before showing that if the secondary curve is rigid it gets worse when we straighten the primary curve.

We practice midline incisions through the window in the plaster cast. We separate the tissue on one side only. The muscular masses are separated on one side in order to operate on the convex side. We go very far out we have to reach the articular facets and then following Hibbs technique we take advantage of the bone and we fill in the channel. If there is room we

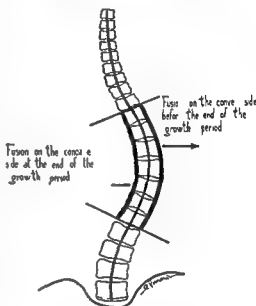


FIG 371 Fusion technique. During growth period fusion must be done on the convex side when growth is ended on the concave side.

put in a rigid graft. We keep the patient in bed as long as necessary following a principle which says that immobility must be maintained as long as necessary.

Treatment of paralytic scoliosis does not come to an end when fusion is solid and when the operation has finished because we must take into account that the bone is elastic subject to pressures and that the deformity forces may continue to act. The difficulty that appears in these cases is pseudarthrosis, the more frequent the more mobile the segment of the affected part of the spine and frequent in all statistics. Both in our own statistics and those of other offices

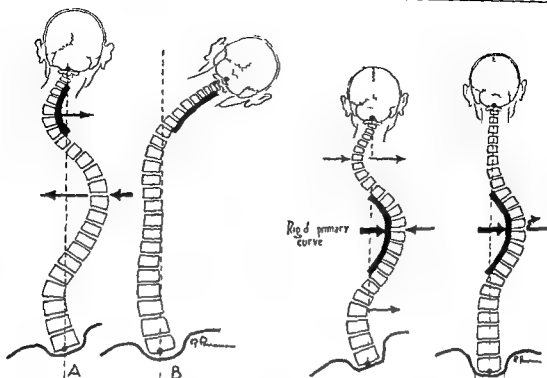


FIG 372 Illustrations showing how the secondary curve if rigid gets worse when the primary curve is corrected

we see that 6 months after the operation even in apparently solid fusion there is a loss of 25 to 30 per cent of the result obtained

We shall now consider other technics. Vertebral osteotomies may be indicated in the fixity of the curve heavy primary curve and here we shall have the advantage of hearing Mr Roaf who no doubt will speak about it because he has developed a technic and has operated in 16 cases applying it

Figure 373 shows resection of vertebral bodies laminae and ribs in order to try to straighten a primary rigid curve. If we take the primary curve and straighten the secondary we shall have a false reduction and scoliosis will continue (Fig 374). In these cases of accentuated scoliosis the osteotomy that Roaf speaks about would be the one that should be practiced. We have very little experience with these cases and we think that this is still in the experimental stage. We have not seen any especially good results. Dr Harrington no doubt also will speak to us about osteotomies but we believe in the biomechanical technics and the rigid

metallic devices placed in order to maintain the spine straight do not seem to us to be entirely satisfactory. We are not sure of the points of pressure. We think that with the lateral curve it can be used but where there is total deformation particularly through rotation we think that technically it must be difficult to place these devices. Nevertheless we consider anything that is done to improve the condition of these patients to be welcome although in the first instance it would seem that the results perhaps would not be satisfactory.

I shall now finish by saying a few words about the most important thing that is the age of the patients who are being treated. As we understand it the most important thing is the growth factor therefore the younger the patient the more severe is the evolution of scoliosis and the more difficult its treatment. The points under discussion are the following:

- 1 Whether the fusion affects growth or not
- 2 Whether or not the fusion in the growing period can produce other curves as serious as those of scoliosis that is secondary scoliosis



FIG 33 (Left) Resection of vertebral bodies laminae and ribs in order to try to straighten a primary rigid curve

FIG 374 (Right) False reduction result from straightening the secondary curve

3 Whether or not operations at any age are truly effective in hold the progressive curves

We think that these are the most important points to be discussed. We believe that in these cases with high progressive curves 100 per cent will have serious scoliosis at the end of growth after a period of observation as indicated by Dr Bennett and his collaborators. If the curve increases we must have the courage as Risser says to reduce the scoliosis and operate on the patients at an early age. In any case we are in the experimental stage in this field. The results obtained are not entirely satisfactory but nevertheless just as we have avoided secondary deformation of poliomyelitis in the limbs we also believe that we should avoid the formation of scoliosis which affects the physical and moral aspects of patients. In any case any operation that may enable improvement of the stabilization of the curves an improvement of the patients moral health always will be helpful.

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## DISCUSSION

Dr HARRINGTON Dr Carlos E Ottolenghi's paper summarizes the present current treatments in the Western Hemisphere and the Continent. It is well for the record that this summary go down in history.

In the past 10 years I have worked relentlessly on a surgical method (1) to improve the surgical treatment of scoliosis (2) to preserve the integrity of the individual and (3) to support the vital functions of the body mainly cardio-respiratory.

I have come to the following conclusions:

1 The etiology of paralytic scoliosis is simply paresis partial or complete of the paraspinal musculature. All other considerations are secondary.

2 Skeletal structure changes are the result of unequal pressures affecting epiphyseal growth.

3 Internal spinal support by mechanical means is possible and indicated.

In the normal growing child the integrity of the spine is maintained by adequate musculature working through symmetrical skeletal tissue. When either of these elements is disturbed potential mal-development is inevitable. By the nature of poliomyelitis and its associated paresis the integrity of stability in the spine is inhibited or lost. The extremes of deformity that may be acquired by the individual are directly related to the amount of instability and the duration of growth potential remaining in the patient.

The ideal treatment must fulfill the following criteria: re-establish and maintain stability of the spine. The present dilemma is how to fulfill these rigid criteria and not disturb in a major way the growth of the spine.

I wish to differ slightly with Dr Ottolenghi that internal fixation of the spine is physiologically and biomechanically possible.

The problems of internal metallic spine fixation with dynamic potentialities are:

1 Can metal be tolerated by the body?

2 Can metallic compression and/or distraction forces be applied and still remain physiologic?

3 What are the forces that are necessary to correct curvature or prohibit its progression?



FIG 3/5 Showing the strut mechanism that has been in the body for 1 year. The top of the photograph reveals the walking hook in place. The lower portion of the photograph reveals the distracting force (rod and hook) unbedded in bone.

4 Are vital functions affected?

5 How may mechanical forces be applied to establish spine stability?

6 Can correcting forces be applied and adjusted with growth?

I would like to give you a slide demonstration (Fig 3/5).

A particular reaction of the spine to existence of metal in its proximity—there is apparently acceptance by the body. The bone is never in contact with metal. There is always fibrous membrane existing between the metallic instrument and the bone.

Figure 3/6 shows a membrane removed between a metallic instrument and the bone. The bone is not present. The membrane in this area is a dense fibrous tissue without polymorphonuclear infiltration. This is a photomicrograph taken of a patient approximately a year ago.

Figure 3/7 shows the bone in contact with this membrane and it eliminates the synovial membrane at the bottom. Note please that the osseous structure is firm and dense and has changed its character to meet the stress that has been put on it.

This is an internal fixation that has been in the body a year and a half. There is no foreign body reaction; there is no rejection by oxidation; there is no rejection by necrosis. This is normal tissue enveloping the various elements of the instrumentation.





FIG 36 Photomicrograph of the synovial membrane that always exists between the metallic instrument and the soft tissue or bone

In the past 8 years more than 50 operations of principle and design have been tried to develop the present concept of the necessary elements to establish correction and stability in a scoliotic spine.

1. There must be a distraction force and there must be a compression force. These forces must work in unison and must have freedom of action to allow for growth and motion yet still maintain the intended function of distraction or compression.

2. On some spines in order to acquire maximum correction in any one sitting a distraction force of over 13,000 lbs per sq in has been applied without deleterious effect. This pressure does not remain constant. Within a few moments or at least hours this force is reduced to practically nothing by release of the soft tissue. These soft tissue elements are classically responsible for the resistance met when distracting forces are applied.

In the past year's work closely related cardiorespiratory function studies on operative cases indicated that all respiratory capacities were improved.

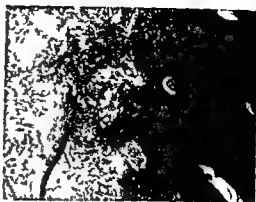


FIG 37 Showing the reaction of the transverse process into more dense bone and the healthy fibrous tissue that exists between the metallic instrument and the bone.

Cardiac studies have revealed that scoliosis shows prolonged mechanical systole which we interpret as a manifestation of cardiac work under uneconomical circumstances. Only measurement will permit us to substantiate the final clinical impression that the correction of the anatomic deformity is followed by regression of the abnormal findings.

In this series of over 20 scoliotic cases operated on under this study starting in July 1956, no case showed vital capacity reduction. No external fixation was utilized in any case. The average hospital stay before release to home care was 17 days. No complications have resulted.

Time and observation will answer questions of the tolerance of this mechanism by growing bone.

True, we have met difficulties in the part of erosion of bone and fracture of the metallic instruments.

In none of the study operations has the heavy used equipment of the latest design had there been erosion of bone allowing loss of distraction force or fracture of the equipment.

In conclusion, I believe that due to the nature of paralytic scoliosis the stability must be reestablished in the spine during the period of growth. It is possible to accomplish this by internal metallic fixation. The method that I present is not considered a panacea and is still in the final development stages. I will show a slide of a recent case (Fig 38) and let this



FIG 38 (Left) Roentgenogram taken on admission and posterior fusion (Center) Posterior fusion (Right) Anterior fusion (3 months postoperative)

method speak for itself. This before surgery duration was 7 hours approximately 35 to 40 minutes were spent in allowing soft tissue to release and distraction force on one side a compression force on the other with final spinal fusion after a modified Hibbs and a long graft. I firmly believe that once the extremely technical factor of application of internal spine fixation is mastered surgical treatment of scoliosis will be the application of internal fixation without or with fusion superimposed fusion being dictated by the age of the patient.

Dr ROAF Dr Olench has rightly stressed the two aspects of paralytic scoliosis—instability and deformity. I have little or nothing to add to his excellent account of the surgical treatment of spinal instability. On the weak portion of the spine has been made stable it is easier for the adjacent portion of the spine to develop compensatory curves the patient holds himself straighter with less effort and fatigue. In addition

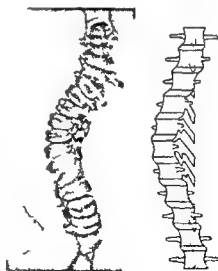


FIG 39 (Left and right) Model reproduction, severe scoliosis at deformity alone without any element of lateral flexion

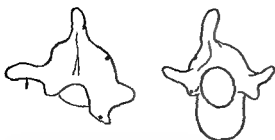


FIG 380 Drawing of a specimen illustrating that the laminae and the pedicles are larger in all dimensions on the convex side at the apex of the curve

tion he has better use of his arms and legs as the proximal limb muscles have a stable instead of an unstable point or origin. His trunk and respiratory muscles also act more efficiently. All

orthopedic surgeons must be agreed on the great benefits of spinal fusion in paralytic scoliosis. Five questions however remain unanswered. First, how extensive should fusion be? Second, at what age should it be done? Third, how can development of deformity be prevented? Fourth, how can established deformity be corrected? Last, after correction and fusion, how can relapse be prevented?

I would like to consider the third and fifth questions first as they are closely linked. The forces causing deformity will, if uncorrected, tend to cause relapse after fusion if the patient is young because the fused spine is young bone and is therefore still plastic. I cannot repeat all the evidence now, but my own researches have led to the conclusion that imbalance of rotation is the most important primary element in the

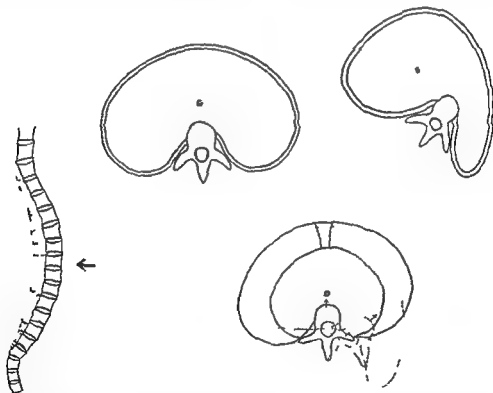


FIG 381 (Left) Illustrating the action of the longitudinal fibers of the erector spinae muscles which make the vertebrae at the center of their span move forward i.e. the kyphosis is decreased or in the lumbar region the lordosis is increased. (Right) Transverse section of normal and scoliotic thorax and the two superimposed. Note that in the scoliotic thorax the vertebrae point sideways. This means (a) that gravity acts asymmetrically in the vertebra and (b) that the normal action of the longitudinal fibers of the erector spinae muscles in pulling the vertebrae anteriorly now moves them antero-laterally and this movement is no longer counteracted by gravity.

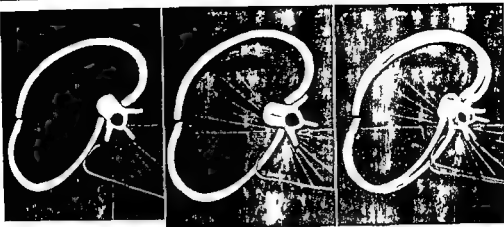


FIG 387 In the scoliotic thorax the head of the rib on the convex side presses posterior to the axis of rotation. On the concave side it presses anterior to the axis of rotation as a result the deformity tends to be increased by the rotatory couple of forces. Pressure on the concave side or pressure on the convex side decreases the deformity.

development of severe paralytic scoliosis. There are of course mild pure lateral flexion types which are easily corrected and present no problem. It is possible to reproduce all the basic features of severe scoliosis by realigning vertebrae in forced rotation deformity without any element of lateral flexion. Figure 379 left and right shows this—you can easily do it yourself and make a model and reproduce severe scoliosis without any lateral flexion. In addition to the intervertebral rotation there is an intrinsic rotatory deformity of the vertebrae themselves which is largely confined to the neural arch. Figure 380 is a drawing showing the hypertrophy of the neural arch on the convex side which is an element in rotation because there is a bigger distance both in the frontal and sagittal planes between the superior and inferior articular facets on the two sides and this would be a bar to reduction.

Once a rotation deformity is established 5 deformative forces inevitably come into action which must tend to increase the deformity or cause relapse after fusion.

1 The first deforming force is the longitudinal fibers of the erector spinae muscle. These fibers normally cause the vertebrae at the center of their span to move forward turning a kyphosis into lordosis (Fig. 381 left).

Figure 381 right shows a normal and a

## EFFECTS OF ROTATION (II)



In neutral position  $AB$  the rotary action of the deep oblique fibres of the erector spinae is balanced  $\angle SAO = \angle S'AO$ .

In the rotated position  $AB$   $\angle SAO > \angle S'AO$  therefore other things being equal the muscles on the convex side act at an advantage.

FIG 393 Illustrating the mechanical advantage of the short deep oblique fibers of the erector spinae muscles on the upper part of the convexity and lower part of the concavity of a scoliotic curve.

scoliotic spine and the two superimposed. Note (1) that the center of gravity lies to the side and does not oppose the longitudinal fibers of the erector spinae muscle. (2) that the vertebra



FIG 384 (*Left center and right*) Posterior fusion in the presence of rotation followed by further growth of the anterior vertebral element increases the deformity although the posterior fusion is quite solid



FIG 385 (*Left*) Before fusion (*Right*) Deterioration after fusion



FIG 386 (Left) Before fusion (Right) Deterioration after fusion

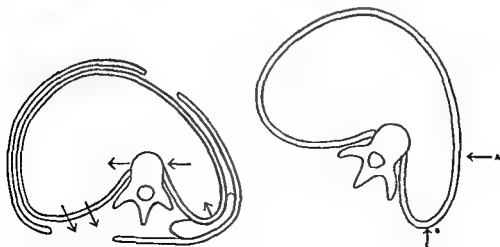


FIG 387 (Left) Diagram illustrating the principle in the use of braces to derotate the scoliotic spine (Right) Pressure on B tends to derotate the spine but lateral pressure at A may increase the rotation

points sideways therefore when the erector spinae muscle contracts it moves sideways unopposed by gravity

2 The point of pressure of the ribs on the convex side is posterior to the axis of rotation on the concave side it is anterior Therefore the rib pressures create a couple of forces increasing the rotation deformity (Fig 382 left middle and right)

3 The center of gravity lying to one side will tend to produce a secondary lateral flexion and unequal epiphyseal pressures (Fig 381 right)

In addition the short deep rotatory muscles of the spine running from a spinous process to a transverse process lower down act at a mechanical advantage on the convex side in the upper part of the curve Normally the two angles SAO SBO are equal In the rotated spine SAO is greater than SBO therefore those muscles act with a mechanical advantage and incidentally they usually are much larger in bulk on the concave side (Fig 383)

Figure 387 is a picture of the rib cage—you see that the ribs on the convex side are posterior to the axis of rotation If you press on the concave side or if you press laterally on the convex side the rotation is increased (Fig 382 left) If on the other hand you pull on the concave side or press posteriorly on the convex side the

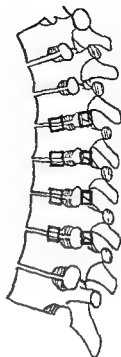


FIG 390 Diagram to illustrate tissue resected at the operation to correct deformity Partial lateral vertebral epiphysiodesis

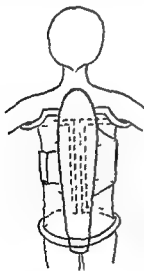


FIG 388 (Left) Brace to exert pressure on the rib hump posteriorly and the opposite chest anteriorly



FIG 389 (Right) Wire traction on ribs on concave side

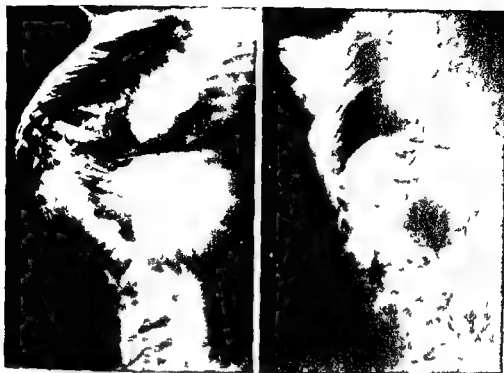


FIG 391 (Left) Before operative correction (Right) After operative correction

rotation is decreased (Fig 387 middle) Figure 387 right is a superimposed picture of the model in two positions

Now the fourth action is the effect of a laminar fusion on a growing spine. The vertebral bodies go on growing; the graft acts as a tether and therefore if the vertebrae are rotated lordosis is increased which gives the appearance of the x-ray increase of lateral flexion and increases the displacement of the vertebral bodies from the midline.

Figure 384 illustrates a girl whom we thought we had corrected but we haven't. There is too much rotation. She was fused. The fusion was sound but she grew and 2 years later this is the deformity.

Figure 385 is another example. This patient also had a laminar neuro-arch fusion in the presence of a deformity. She grew and the result was this. Spinal fusion in the presence of rotation can have a harmful effect in the growing child.

Figure 386 illustrates another case. We fused

in the presence of rotation the greatest mistake I must confess and here she is 7 years later—you see the vertebral bodies have gone on growing and the fused area has not.

For those reasons I believe that correction of the rotation deformity—it necessary by operation—should be undertaken in every case of severe progressive or fixed paralytic scoliosis before laminar fusion is performed.

Figure 387 shows the principle of conservative correction. You ought to press on the ribs posteriorly not laterally—press on the opposite side of the chest anteriorly and pull on the ribs on the concave side to cause a derotation force. Now this can be accomplished by braces. This is a simple sort of brace—the ordinary Thomas brace with pads pressing on the convex ribs posteriorly and the concave ribs anteriorly (Fig 388). You can do it with different types of plasters—there are many ways of doing it. You can do it by operation putting wires around the ribs and pulling on them. It is quite easy (Fig 389). You see this boy playing about



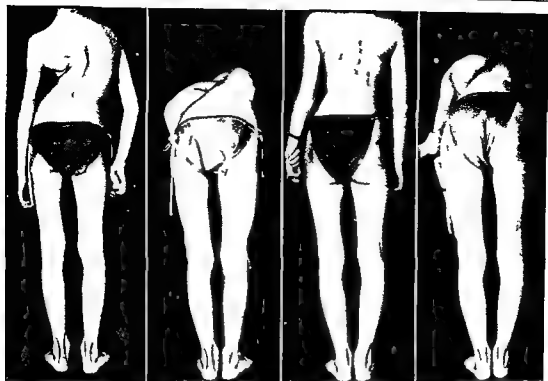


FIG 392 (Left) Before rib resection (Right) After rib resection



FIG 393 (Left) Before partial resection of scapula  
(Right) After partial resection of scapula

6 weeks after operation. In extreme cases you may have to resect portions of the interlaminate joints and intercorporeal joints where fixed bony deformity is present (Figs 390 and 391 left and right).

As to age of fusion—I am reluctant to perform a posterior fusion below the age of 12. Under that age I rely on plasters or braces and advise the patient to use crutches. If the deformity forces are very strong I perform lateral vertebral epiphyseodesis (Fig 390). When I do a posterior fusion I fuse the whole of the major curve and 1 or 2 extra vertebrae at each end (Fig 391 left and right).

Finally in the older age group I believe that there is a place for cosmetic procedures or resection of ribs and transverse processes. Before rib resection you notice the rib hump afterward

you can hardly see it. We haven't altered the curve. We improved her appearance and she was very grateful (Fig 392 left and right). I have found that this pleases the patients in the older age group.

Another form of cosmetic operation is resection of the upper part of the scapula. The girl in Figure 393 had her scapula stuck up so much that she couldn't hide it even under the coat she wore. After resection of the upper part of the scapula she looked practically normal from the front when suitably clothed.

Finally I would like to summarize the key to paralytic scoliosis—the study of the reforming forces—and I would like to say how much I appreciated the honor of being asked here to discuss Dr Ottolenghi's comprehensive and balanced review of an important and difficult subject.

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## *Functional Surgery in Patients with Severely Paralyzed Upper Extremities*

DR VERNON L. NICKEL

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When treating patients with severely paralyzed upper extremities the usual concepts of assessing improvement or judging the success of operations in terms of the normal hand should be discarded. Otherwise many people with most distressing disabilities will be denied procedures which proportionately would be of great benefit but when considered in comparison with normal function the gain would not be great. Such relatively small improvements in the severely paralyzed offer a tremendous step forward in their functional rehabilitation.

Extensive time motion studies of prehension show that the major portion of our upper extremity activity is in a rather small area extending from the waist to the face. Thus in planning mechanical substitution or surgical reconstruction of these upper extremities we should plan to center function in this area. Again if goals are set in terms of normal function—for example elevation of the arm—then because of obvious inability of the surgeon to supply such range the patient will be denied anything.

When considering supplying mechanical or surgical benefits to a patient with severe paralysis of the upper extremity we should keep constantly in mind the area and type of activity that is most desirable for this type of patient.

A high percentage of patients with severe paralysis of the upper extremities have required or even may still require mechanical respiratory assistance. With a low vital capacity this type of patient commonly is denied the benefit of well planned surgical procedures because of the apparent severe risk attendant with any surgery. At the Rancho Los Amigos Hospital Respiratory Center for Poliomyelitis surgery on patients with severe respiratory paralysis has been done for over 3 years. During the recent 12 month period from March 1955 to March 1956 a total of 264 patients were operated on. 25 per cent required respiratory equipment prior to surgery

and 52 per cent required respiratory assistance during the postoperative period. There has been no operative or postoperative mortality despite the fact that very extensive surgery has been done. Only a few relatively minor complications have occurred. It is our opinion based on this experience that the severity of respiratory paralysis should be no deterrent in the planning of reconstructive surgery when adequate mechanical respiratory assistance and personnel trained in the use of this equipment are available.

Another important consideration in overall upper extremity activity is the correlation of respiratory tolerance with the amount of muscular energy required to perform work. We have found patients that are so borderline as far as respiratory capacity is concerned that they severely curtail the use of their arms because of the increased breathing effort required. Thus in this type of patient respiratory equipment in directly will enhance upper extremity usefulness greatly.

Increasing interest is being shown in the attempts at mechanical replacement of totally lost function or mechanical reinforcement of less than total paralysis. As one works with this type of problem it becomes apparent that whenever possible mechanical devices should be replaced by surgical procedures but that many surgical procedures in the severely paralyzed would be impossible and would not be considered without the knowledge that mechanical apparatus of different types for these irreplaceable functions is available. For example if operative reconstruction of a hand is contemplated on an otherwise flail upper extremity, elbow and shoulder stability and motion can be provided by mechanical means. The next few years will see a definitive improvement in this type of apparatus.

Each year surgical procedures that formerly would have been considered quite radical are

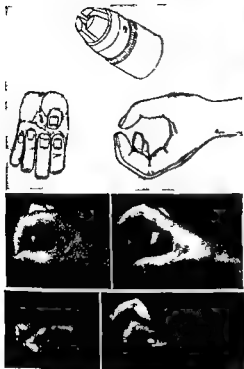


FIG. 394 (Top) Illustration of flexor hand's muscles. A mechanical chuck. FIG. 395 (Bottom) (Upper) A good flexor hand's hand. (Lower) Some hand's hand.

presented with a high proportion of good results. Even more striking is the proportion of cases in the series of implantation of upper extremities that are being considered for surgery. Time permits giving credit to the many pioneers whose fundamental contributions have made this possible.

When any transfers are considered a full range of motion of the joints involved is an absolute prerequisite. The more severe the extent of paralysis the more important it is to adhere closely to this principle. If splinting casting and physical therapy do not achieve this surgical release is required before further definitive work is done.

The focus of upper extremity function is the pinch and grasp produced by some type of prehension. Unless some means is provided for this function will be motivated. Obviously substitution for pinch can be made by devices that



FIG. 396 Illustration of the function of a flexor hand's splint.



FIG. 397 Wrist extends on using substitute to common finger extensors which permit to transfer entire weight without function.

are attached to a splint or hand but it must be emphasized that whenever possible a means should be provided to pinch. Of course the complete substitution for hand prehension is the hook used by amputees as a matter of necessity. Few patients with severe paralysis will function well with a hook and the hand itself should be used to supply this pinch. The reason is that the most important factor of sensation becomes more dominant in the weaker hand. In contrast



FIG 398 (Left) A hand in useful function after supination release

FIG 399 (Right) Mechanical splinting to make use of remaining supination power

an amputee usually has adequate strength in the stump and a normal opposite extremity and with this a degree of functional sensation through the prosthesis can be obtained. This is not so in a patient with severe paralysis who has to concentrate maximal effort in providing a certain small amount of active motion and by doing this sensation through mechanical apparatus is largely lost.

Surgical methods of providing active pinch fall in 3 main categories:

1. Tendon transfers of which opponensplasty, restoration of finger flexion or extension and so on, are most basic and in properly selected cases attendant with a very high percentage of successful results. These now well known procedures will not be discussed further.

2. Tenodeses, active and passive, are very useful and should be used when active tendon transfers are not feasible. These provide active prehension or in certain instances may be used to provide a passive hook. Dr. Tyler will present these in his discussion.

3. Flexor hinge. The employment of this prin-

ciple is also restricted to those hands in which active tendon transfers are not available and the choice lies between tenodesis and the flexor hinge hand. The concept of the flexor hinge hand may be combined with tenodesis.

Extensive research of the function of the hand has demonstrated that the major portion of our prehension is done through a mechanism similar to a simple chuck used by mechanics. Thus we believe our major effort should be directed to replacing a chuck type of prehension (Fig. 394).

A flexor hinge hand is one in which the thumb is stabilized in opposition. The interphalangeal joints of the index and long fingers and occasionally of the ring finger are also arthrodosed in a position of function. The only remaining motion in this hand is in the metacarpal phalangeal joints which now act like a hinge—therefore the name flexor hinge hand.

The flexor hinge hand will give surprisingly good function when muscle strength is minimal. A total of 2 functioning muscles which may be quite weak will provide active prehension—1 for flexion and 1 for extension. Flexion is provided without further transfer if a profundus, sublimus, interosseus or lumbrical muscle is present. When no active extension is available without or with transfer, mechanical extension can be provided and if no muscle power at all is available the concept of the flexor hinge will provide prehension with a minimum of splinting. Figure 395, upper and lower, illustrates such a hand.



FIG 400 Flexorplasty utilizing a transfer of weakened forearm muscles showing range of motion obtained and the principle of active tenodesis to prevent full extension

In this hand the wrist is stabilized by its remaining power. When this power is not sufficient a splint is used. The thumb is held in a satisfactory position of opposition by bone block and interphalangeal fusion. Interphalangeal arthrodesis maintains position of the fingers and the only motion present in the hand is MP flexion and extension. A true type of chuck pinch is obtained (Fig 396).

Prior to any surgical procedures the hand is splinted in this position for a period of time so that the patient knows what function he will have and the surgeon determines by trial and error the optimum position for maximum efficiency.

Arthrodesis of the wrist should not be done until prolonged splinting has demonstrated that this procedure will not cause loss of some desired function. Of particular importance is the fact that tenodesis procedures depend on wrist motion and if arthrodesis is done any such procedures cannot again be considered. Active transfers to gain wrist extension are quite effective of which transfer of a sublimis is probably one of the most useful (Fig 397).

If either isolated pronation or supination remains active rigid rotary deformities are very common. These may require surgical release if prolonged casting will not correct them. Full supination deformities particularly must be



FIG 401 Flexorplasty with good forearm muscles and pooriceps—an ideal situation for this procedure

brought to a pronated position before the hand can be functional (Fig 398).

When one component of forearm rotation is lacking in the presence of a free range of motion it may be supplied mechanically (Fig 399).

Elbow function will be considered next. Again if at all possible surgical procedures should be done to gain active elbow flexion. Flexorplasty results in remarkable improvement. Not only is active elbow flexion obtained but the transferred muscle across the elbow joint acts as an active tenodesis preventing full extension and thus gaining tremendous mechanical advantage for the weakened muscle to flex this elbow (Fig 400). The triceps muscle is a good transplant to gain elbow flexion (Fig 401).

We would like to emphasize at this point and by this patient, another consideration that is so very important to keep in mind in doing tendon transplantations. In most instances two gains are made: (1) to remove a deforming force and (2) to gain function. In this case the triceps was relatively overactive. Any attempt to use the hand was greatly impaired by the triceps forcibly extending the elbow. When the



FIG 402 Triceps flexorplasty

triceps was moved to flex the elbow not only was active elbow flexion gained but hand function was indirectly enhanced as well (Fig 402)

When no active transfers are available to gain elbow flexion the elbow locking device is most useful. Flexion force can be enhanced by providing springs or rubber band assists (Fig 403)

Without shoulder stability elbow flexion is of limited value because as the elbow flexes shoulder extension must be blocked so that the hand may remain in the useful range area. We have not had any experience with active transfers to stabilize a shoulder. This is because in patients who have had severe respiratory paralysis there seldom are available muscles of suffi-



FIG 403 The mechanical locking elbow to provide elbow stability

cient strength for transfer about the shoulder girdle. However shoulder arthrodesis we believe is of the utmost importance and has not been used sufficiently. Criteria have been established for shoulder fusion which we think are too rigid. Obviously the more complete scapular control results in more motion but even a moderate degree of scapular control will provide excellent stabilization and a reasonable range of functional motion of the humerus. Also we believe that it has been customary to fuse the shoulder in too wide abduction in order to gain elevation which as has been pointed out is of limited value. Wide abduction interferes with comfortable turning in bed and with crutch walking but most important the hand is not in the optimum position (Fig 404)

Mechanical apparatus can be used to provide shoulder stability as illustrated in Figure 405



FIG 404 (Left) Shoulder fusion with weak scapular muscles illustrating range and stability obtained

FIG 405 (Right) Mechanical shoulder stabilization

As we consider the over all picture of upper extremity function we must keep in mind the consideration of trunk stability by casting by abdominal fascial transplants and spinal fusion of scapular control by interscapular fixation and of neck positioning by bracing and fusion—all of which very directly affect the efficient use of the upper extremity in the type of patient we are considering. Time prevents elaboration of this point.

In summary 4 points have been emphasized.

1 In considering the severely paralyzed upper extremity we should assess our results in the

light of improvement gained for this particular patient and not in terms of normal function.

2 The severity of respiratory paralysis does not preclude major reconstructive surgery.

3 The combination of mechanical apparatus and reconstructive surgery is an effective means of supplying basic upper extremity function with emphasis on removing apparatus by tendon or even muscle transfers or arthrodesis when ever possible.

4 The flexor hinge hand is a means of providing a tive prehension with minimal muscle power.



## DISCUSSION

**DR CAMPOS** I am very happy indeed that so worthy and well experienced an investigator as Dr Vernon L. Nickel was selected for the presentation of the extremely difficult problem of reconstructive surgery in severely paralyzed upper extremities

His paper was excellent and realistic. There is very little to add but many points to focus the spotlight on to emphasize its practical and valuable aspects

The point that impresses one most is the safety with which operations can be performed on patients with severe respiratory involvement which quite often is associated with extensive paralysis of the upper extremities. In this particular field nobody in the world can match the experience of the American surgeon who works in the famous Respiratory Centers. We just cannot have the same experience because most of our patients with spinobulbar type of polio do not survive the acute stage to undergo later reconstructive surgery due to lack of organization and trained personnel

Certainly one must agree with Dr Nickel that the principles and techniques for tendon transfers are rather well established and that the proportion of good results are very high in experienced hands

A reasonably useful upper limb depends very much on a certain amount of shoulder stability. Arthrodesis of the shoulder is without doubt an excellent operation and sometimes it is worth while to perform before adolescence since the risks of developing a mild scoliosis are far outweighed by a better functioning shoulder. However if after the ravage of the acute stage some muscles are spared to permit an attempt to obtain the active stability of the shoulder joint by the usual methods available they should always be tried because these operations do give quite satisfactory results and besides they do not interfere at all with the possibility of late arthrodesis in case of failure

Among all the operations proposed for paralysis of the deltoid I have had a very gratifying experience with the Leo Mayer transplantation of the trapezius, a muscle seldom touched by infantile paralysis

I recommend this operation when I feel it is indicated and I am not too rigid as to the criteria that all the other muscles of the shoulder should be normal. In the presence of paralysis or marked weakness of these muscles with subluxation of the scapulohumeral joint a sufficient scapulohumeral stability is obtained with the Nicola tenodesis thus permitting the transplanted trapezius to work and restore to the shoulder some power of abduction

I have found that the resection of the external third of the clavicle and part of acromion eliminates the hindering hump of the shoulder, improves the direction of pull of the trapezius and also gives the shoulder a better rounded appearance

Now I am going to show you a couple of cases

Figure 406 left shows a little girl who had complete paralysis of the deltoid and who uses all the other muscles of the shoulder girdle

Figure 406 center and right are photographs made after the trapezius transplantation to the deltoid showing the amount of limitation of the shoulder movements

Figure 407 top left and right are photographs of another patient before and 9 years after the operation transplantation from trapezius to deltoid in a case of complete paralysis of this muscle and with a severe scoliosis. The roentgenograms show the range of motion obtained by the operation

There is one last point I would like to make some remarks about. The combination of mechanical splinting and reconstructive surgery to improve the function of the upper extremity was very well illustrated and its possibilities at the present time clearly demonstrated by Dr Nickel

Investigations and experiments are being made not only in the field of external mechanical devices but also in the field of endoprosthesis where I believe exists a reasonable basis of hope for the future

Since the early experiments of Lambotte the famous Belgian surgeon to replace the lost function of muscles by internal elastic devices a great deal of work has been done along this



FIG. 406 (Left) Complete paralysis of the left deltoid remaining muscles of the shoulder girdle normal and normal relationship of scapulohumeral joint (Center and right) Transplantation of the trapezius to the deltoid End result 8 months later Excellent stability of the shoulder and satisfactory power of abduction

FIG. 407 (Top left) Extremely accentuated paralytic scoliosis Total paralysis of the right deltoid The outer third of the clavicle and part of the acromion were resected to improve the line of pull of the transplanted trapezius (Top right) End result 9 years after the operation Perfect stability of the shoulder and a reasonable power of abduction (Bottom) Roentgenograms showing the range of motion of the scapulohumeral joint after resection of external extremity of the clavicle and part of the acromion





Fig 408 Shoulder fusion

line In a recent paper from the Norbaha Institut John Sevastikoglou makes a very interesting review of what has been done in the field of muscle substitution by elastic endoprosthesis and shows the results of his own investigations

We all know and agree that the great hindrance to the replacement of a joint by an endoprosthesis lies in the fact that we have materials rigid enough but not with the elasticity of cartilage to work as a buffer between the frictional surfaces of a joint

On the other hand the replacement of muscles will depend of course on the discovery of coil springs made of elastic materials rigid enough to stand the wear and tear of time and action

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Sevastikoglou J An experimental attempt to replace muscle function by elastic endoprosthesis Acta orthop scandinav 26 161 1957

DR EYLER One need only to have attended previous conferences to realize how difficult it is to present something new and at the same time have it interesting and instructive Dr Nickel is to be commended

In the past many of us have been justly critical of the mechanical and even the mechanosurgical approach to rehabilitation of the severely

paralyzed upper extremity Most of the function arm braces or orthoses have been bulky unwieldy expensive and more important rejected by patient and parents alike In his thoughtful and instructive presentation at the 1951 Conference Dr Seddon of England voiced the opinion that There is no apparatus for the permanent control of the wrist elbow or shoulder which approaches in usefulness what can be achieved by surgical intervention

Our thinking has been mollified and our practice modified by recent advances in orthotics under the leadership of Drs Shottstaedt Nickel and others Team approach to evaluation prescription fitting and training is proving to skeptics myself included that the use of orthoses on the severely paralyzed upper extremity is an integral part of the rehabilitation of this type patient The motivating mechanism of the Heidelberg arm brings an exciting new facet to the field of orthotics Case selection is a critical factor but anyone who resists employment of such apparatus on grounds of impracticability will sooner or later be guilty of disservice to his patient The goal of course is to eliminate all external assistive and supportive devices whenever possible but the mechanosurgical approach as presented by Dr Nickel is sound

I am always irritated by a discussant who in effect ignores the principal issue and proceeds to present a subject of his own However on this occasion I have been given poetic license by the author

Arthrodesis and tenodesis are the most potent weapons of salvage in surgery on the severely paralyzed upper extremity In most instances arthrodesis is an irrevocable procedure used to stabilize a joint for functional and occasionally cosmetic reasons By arthrodesis one is often able to transfer the allegiance of remaining muscle motors to more distal segments of the extremity Shoulder fusion is a classic example (Fig 408)

Tenodesis is a simple surgical procedure that is both revocable and alterable By definition tenodesis is tendon fixation or suturing of the proximal end of a tendon to bone Tenodesis can be flexor extensor intrinsic or opponens in type Extensor tenodesis of the hand is seldom necessary When the wrist is flexed the hand opens automatically The result of opponens tenodesis often is not worth the surgical effort

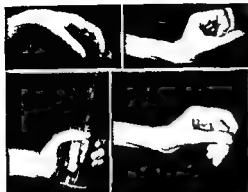


FIG 409 Patient with two tendon hand

Many methods have been tried but in the severely paralyzed upper extremity bone block opponens gives a uniformly better result. Flexor tenodesis provides an excellent method of salvaging function in the flail or partially paralyzed upper extremity. The strength of ordinarily non-transferable muscle motors can be utilized by tenodesis of the antagonists. For example a fair (50% of normal) wrist extensor ordinarily not considered transferable can be made to serve the dual purpose of wrist stabilization and power for motivation of a tenodesed antagonist. In Figure 409 top right and left and bottom right and left we see a patient who has a two tendon hand. The forearm and hand are essentially flail except for power in the long wrist extensor and the brachioradialis. By extensor flexor intrinsic tenodeses and transfer of the brachioradialis for opposition this hand has improved cosmetically and functionally. In the Armed Services it was customary to say that it by reconstructive surgery one was able to give the patient sufficient pinch to hold a quarter and enough grasp to hold a bottle of beverage he was ready for duty.

By flexor tenodesis a completely flail hand can be converted into a useful hook with all important sensation. Figure 410 shows the hook and Figure 411 the flexor tenodesis hook in use. Intrinsic tenodesis will reduce or prevent clawing of the fingers. Intrinsic tenodesis can also be used in conjunction with active transfers. Limited experience with flexor tenodesis of the elbow has been encouraging and in most cases is better than arthrodesis or posterior bone block at the elbow. The principle of tenodesis can be



FIG 410 Completely flail hand converted into a useful hook



FIG 411 The flexor tenodesis hook in use

applied by anyone with a good knowledge of surgical anatomy and of course a true appreciation of the functional problem.

Day by day improvement of the respirator has proved to be a two-edged sword. In the past 5 years there has been a marked reduction in the mortality rate of patients severely involved by poliomyelitis. At the same time we have been presented with problems for which experts in rehabilitation have little precedent in treatment. Laterally to get something from nothing it has been necessary to reassess the surgical mechanism



FIG 412 (Left) Pull on the extensor digitorum communis tendon causes dorsiflexion in the MP joint but incomplete extension in the interphalangeal joints (Center) The same as at the left but at the same time pressure in volar flexion on the I phalanx—thereby the interphalangeal joints extend with good force (Right) Tendon in the flexor digitorum profundus inserted in a bore hole in the proximal part of the I phalanx pull on this tendon causes volar flexion in the MP joint—thereby extension in the interphalangeal joints through the extensor digitorum communis

cal and mechanosurgical approach to rehabilitation of these severely involved patients. By combining established procedures, experience in genuity, trial error and research, Dr Nickel has improved upon this concept of treatment. Under the leadership of the National Foundation for Infantile Paralysis, others are being stimulated to assay and improve on the techniques so capably presented by Dr Nickel.

DR THO JASEN: The paper presented by Dr Nickel has been a great inspiration to me. The fact that Dr Nickel is able to present material of this volume proves that his experience is really great. It is impressive that he has been able to operate one patient a day of this type without mortality. We have been afraid of operating when patients have had respiratory paralysis and it is always a psychological complication when we want to do extensive surgery on a child whose life has been saved by respirator treatment. Dr Nickel's results may support us

in our task of persuading the parents to do surgery on their child.

A very important function is the pinch. The opponensplasty is a well known procedure but is not always fulfilling the goal. I think the function of the abductor pollicis longus and the extensor pollicis longus is very important as basic function for an opponensplasty with the ordinary activation by a crossing tendon from the ulnar edge of the wrist. When the function of the thenar muscles is deficient there may be a tendency to flexion in the interphalangeal joint—giving a secondary dorsiflexion in the MP joint. The way in which I have tried to overcome this deficiency in extension of the interphalangeal joint is by transplantation of the crossing tendon to the aponeurotic expansion toward the tendon of the long extensor of the thumb. The tendon of the flexor sublimis is split to a point close to the MP joint of the thumb—one half is conducted below the long

TABLE 181 POLIO EPIDEMIC IN DENMARK, 1957-1953

	AGE 0-6	6-15	15 YEARS
Total number of patients with severely paralyzed upper extremities	88	45	100
Treatment for respiratory paralysis with or without tracheotomy	36	23	68
Operations			
Hands (activation)	16	19	21
Elbow (activation of flexion)	11	2	3
Arthrodesis of wrist		1	
Rotation-osteotomy of the forearm		1	

extensor to the ulnar sesamoid bone. Here the tendon is looped around the tendon expansion and fixed. The other half of the sublimis tendon is looped in the same way around the tendon expansion close to the radial sesamoid bone—the tension must be regulated to give good opposition of the thumb and extension in the distal joint.

Paralytic first dorsal interosseus is a problem—the different types of tendon transfer to the lateral band of the dorsal aponeurosis has not been really effective in my hands.

The function of the intrinsics is to volarflex the M P joint and to extend the interphalangeal joint. If one removes the tendons of the intrinsics and pulls the extensor communis the M P joint will hyperextend and the interphalangeal joints will stand slightly flexed but if one presses the proximal phalanx in a little volarflexion the two interphalangeal joints will extend with good force. In the case of paralyzed intrinsics I have taken the sublimis tendon from its insertion and have transplanted the split tendon to the proximal third of the proxi-



FIG. 413 (Left) Patient with severe paralysis in the interosseus muscle and the opponens muscle (right hand). (Right) Left hand was primarily as the right. The patient has opponens plastic and transplantation of the flexor digitorum sublimis to the 1st phalanx. The patient thereby has a forceful pinch function between the index finger and the thumb.

mal phalanx through a fine bore hole from the radial side. The two parts of the tendon are sutured together on the ulnar side. It gives a forceful flexion in the M P joint and at the same time by pull of the long extensor a forceful extension in the two interphalangeal joints—thereby giving the index finger the pinch function (See Fig. 412 left center and right and Fig. 413 left and right).



*Group and Home Care of Patients  
With Respiratory or Extensive Paralysis*

FRIDAY AFTERNOON, JULY 12 1957  
(This Session Convened in the Salle du Conseil General)

*Chairman*

DR CINO FRONTALI  
University of Rome  
Rome

*Speakers*

DR KENNETH S LANDAUER  
National Foundation for Infantile Paralysis  
New York

DR JOHN E ATTELDT  
Rancho Los Amigos Hospital  
Hondo California

DR JOSEPH BLUNTON  
Coldwater Memorial Hospital  
New York

MISS LUTH LOCHER  
University Hospital  
Ann Arbor

*Discussants*

DR KARL HARTVIGSEN  
Ullevål Hospital  
Oslo

DR KENNETH S LANDAUER  
National Foundation for Infantile Paralysis  
New York

DR J PEJNE  
Epidemiologiskhuset  
Stockholm

DR WENDOLYN SHEPHERD  
Argentine Polio Foundation  
Buenos Aires



# *A National Program of Respiratory and Rehabilitation Centers*

DR KENNETH S LANDAUER

For the most part such patients as we shall be discussing in the next hour formerly would have died. Instead they now survive with disabilities that pose a tremendous challenge to us all. Having kept them alive we must learn how to provide for them again the opportunities to live as citizens in a free society the opportunities to be useful the opportunities to be happy. The program we will discuss was designed to meet this challenge.

The National Foundation is a nongovernmental voluntary health agency. It is supported by the people of the United States to conduct the battle against poliomyelitis. The Salk vaccine is the result of its research program and promises now to reduce the paralytic consequences of the disease but in our country there are still many thousands of patients stricken in former years and unfortunately many thousands of these are very severely disabled. It is the obligation of the National Foundation for Infantile Paralysis to help them.

In 1949 we conducted a study of the needs of such severely disabled patients. In 1950 we initiated a program to establish special respiratory and rehabilitation centers throughout the United States. We now have 15 of these in operation established at teaching hospitals of 15 medical schools. They range in size from small units of 15 patients to a large center with 160 and total something like 500 treatment and rehabilitation beds for very severely disabled polio patients.

In Figure 414 the large triangles show the distribution of the centers of which I speak. The smaller dots will give you an idea of the density of the spread of paralytic survivors of poliomyelitis throughout the United States. They follow roughly the distribution of our population.

In this brief paper we summarize the objectives of this program the concepts guiding its development some of its accomplishments and the implications medical and social and economic derived from our experience of some 7 years.

As to needs purposes and objectives—the nationwide study in 1949 revealed a growing number of catastrophically disabled survivors hundreds chronically dependent upon respirators for maintenance of life itself. These hopeless patients were scattered over hundred of hospitals where little could be done for them and where they seemed destined to linger through a tragic terminal existence.

Factors contributing to this increasing load of seriously disabled patients were the rising incidence of paralytic polio and the rising age in incidence in the United States where poliomyelitis is more severe in the older age groups. For example two thirds of our patients with respiratory paralysis are young adults. Improvements in medical management of acute life threatening forms of poliomyelitis in the United States resulted in a remarkable reduction of case fatality rate which fell from 65 in 1949 down to 3.6 in 1956. This explains the rapidly increasing number of tragically paralyzed survivors.

It became increasingly important to do something practical positive and a little more intelligent about this increasing load of patients. Patients imprisoned in respirators and hospitals represented a human need but there was a scientific and economic need as well.

The National Foundation underwriting the care of patients who cannot afford care themselves found in 1950 that it was costing over \$3,000,000 to maintain the relative handful of 400 such respirator patients on a custodial kind of care scattered in many hospitals throughout the country. A positive approach to this problem was to concentrate these patients in special centers help equip and staff the centers especially for the purpose of conducting care research and teaching and to demonstrate the ever improving patient care that would result. This was the genesis then of the center program.

Let me discuss the basic concepts and philosophy for a moment. The National Foundation invited several medical school teaching hospitals to accept groups of these chronic respiratory

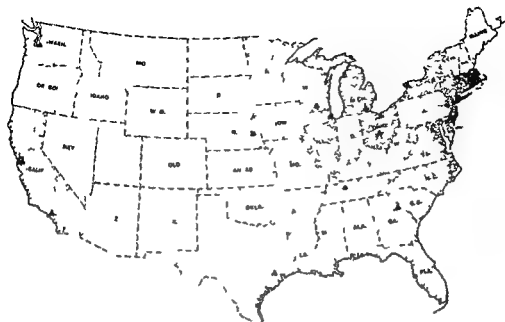


FIG. 414 Total number of reported poliomyelitis cases by state 1947-1956. The triangles denote regional respiratory and rehabilitation centers. Each dot represents 100 reported cases of polio.

patients. The plan was to demonstrate the value of the multidisciplinary concept of care to enlist and train allied personnel to encourage dedicated clinical and administrative leadership and to educate professional groups on the value of experimentation in the problems of the chronically hopelessly ill.

The first two centers were established in 1950 in Boston and Houston. Others have been added gradually as determined by the success of the program and new regional needs. Annual supporting grants from the National Foundation have provided funds for key staff personnel, special equipment and supplies, institutional administrative and overhead expenses. In addition, the chapters of the National Foundation have paid for the actual cost of the hospital care of these patients and for adaptive equipment. Thus we had teams—physicians, nurses, physical and occupational therapists, medical social workers, psychologists and others—working with patients grouped in centers who had everything they needed in the way of equipment and other facilities to pursue research and to do the kind of care they wanted to do free of concern

either on the family's or the hospital's part about payment of the cost for such care. The accomplishments of this program reflect I think the excellence of the medical directors of the center and their associates. The medical director at his best has served as the leader of a democratic group which includes of course the patient.

The center directors regard multiprofessional staff conferences as indispensable in the planning for total care. Doctor, nurse, physical therapist, occupational therapist, medical social worker, psychologist, orthotist, engineer, vocational counselor and teacher are included. Each plays an important role in finding solutions for complex problems. Through these conferences the staff can reach agreement on total patient-care objectives and thus maintain a consistent attitude toward the patient and family.

Underlying the center's program is the concept of comprehensive care which helps to take into account the patient as a whole human being. The physical, mental, emotional, spiritual, social and vocational problems are all interrelated. The center team is concerned about all

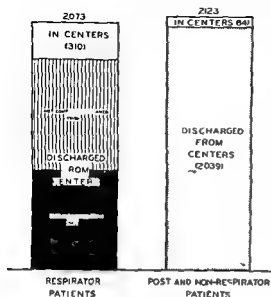


FIG 415 Status of patients ever treated in respiratory rehabilitation centers from 1950 to September 1956

these values and should be able to provide or obtain every kind of help needed. No single professional discipline is sufficient. The concept of comprehensive patient care implies that all members of the team will search actively for new and better solutions to the problems of the severely incapacitated patients. The results of such an attitude are already evident. In 6 years of operations this expanding program of respiratory and rehabilitation centers has freed thousands of such hopeless patients from meaningless incarceration in hospitals. It has helped them achieve greater degrees of self-sufficiency and independence than had been thought possible.

Figure 415 shows that from the inception of the first center in 1950 to September 1956 4 196 patients had been admitted for rehabilitation and 3 802 had been discharged. All of them had severe widespread paralysis from poliomyelitis. Of the 2 073 admitted in respirators 1 763 had been discharged. 845 of them completely weaned from respirator aids. The most significant fact the greatest accomplishment of all was that of 918 patients whose lives were permanently dependent upon artificial respiration equipment 92 per cent (or 845) had been released from existence in hospitals to return to fuller lives at home.

The average time for rehabilitation of such respiratory patients in the centers was 7 months the average cost approximately \$7 000 per patient. These patients had been in other hospitals for a year or longer prior to center admission and had become relatively fixed. The 7 months period of rehabilitation is indeed a great achievement.

In the efforts to rehabilitate respirator patients the majority of whom were essentially quadriplegic the centers also have developed successful new techniques and methods that are of benefit to the nonrespiratory severely disabled patients as well.

Figure 416 shows that in 1949 at the time we first surveyed the number of respirator patients in the country we had a relative handful 451 of whom only 52 had been able to return home. Through the successive years we find a growing number of respirator patients added to greatly in 1957 and 1953 by large epidemics. The white column in each instance indicates the number who have been helped to return home even though still needing respiratory equipment. The last column shows that by January 1957 there were still 579 chronic respirator patients still in hospitals but I would like to point out that as of June 1957 a great many of these also have been successfully rehabilitated and returned to their homes. Thus in the United States today through intensive efforts of these special centers in research and multidisciplinary team care we have found some solutions to problems of the chronic respiratory patient and we have learned how to be of more assistance to the nonrespiratory severely disabled patients.

Now let's talk economics for a few minutes. If we accept the center directors' estimate that about 40 per cent (1 500) of the 3 802 patients discharged would otherwise have continued to require domiciliary care in hospitals or nursing homes for the remainder of life we come to some appalling statistics. It is worth speculating on the financial cost of giving merely custodial care to this type of so-called hopeless patient who might as has been demonstrated through comprehensive care be returned home.

Assuming conservatively an average life span of only 5 years for these patients and a per diem hospital charge of about \$70 it can be seen that the total cost of providing mere custodial care

runs into many millions of dollars. We have estimates indicating that dollars spared by this program from expenditures in needless custodial domiciliary hospital care amount to almost 50 million. Thirty million dollars are better dedicated to research and teaching than to unproductive custodial type of hospital care.

There are costs for caring for many of these patients at home but these are considerably less than in the hospital, ranging from one tenth to one quarter of what hospital costs would be. Even if the more important human values are disregarded it is evident that the millions of dollars spent on unnecessary custodial care is a serious waste. Also it is important to point out that the medical responsibility of the center does not stop when the patient has been discharged home. In the next paper Dr Afillet will indicate the scope of the program in one such center.

Another fact of economic significance which we discovered in this program and is important also in every type of chronic illness is that the later in the disease that the patient is admitted for rehabilitation the harder the job the longer the period needed the less the benefits and the greater the cost. Traumatic and preventable deterioration or dissolution physiologically and emotionally starts at the onset in the acute illness in this as well as other diseases. Much of this damage becomes relatively fixed in polio in 4 to 6 weeks. If comprehensive services can be given from onset of the acute illness only one third as much time and money are consumed and the functional end results are much better.

It is important to note that in granting funds to help establish the centers the National Foundation for Infantile Paralysis has provided no stereotyped pattern for their operation. They

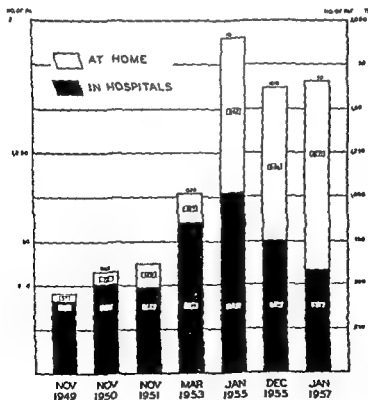


FIG. 41C Number of respirator patients in hospitals and at home based on natural surveys 1949-1957

develop their own programs. However, the National Foundation staff has occasionally been helpful with guidance and advice.

It is of interest to note that all of the original medical directors and most of the key personnel on their teams have remained a part of the program and have become more enthusiastic each year. This is a remarkable record for programs of care for the chronically ill have been notably difficult to staff with well-qualified persons. One suspects that the reason is not merely the support of and freedom to do research and teaching nor the provision of personnel, facilities and other resources to do whatever can be done to help these patients. Nor is pity the guiding motivation. The explanation for this interest would seem to reside in the stimulating effect on the staff of the patients themselves, the human values of their achievements, their accomplishment of seeming impossibilities despite the enormity of their physical deficits and the tremendous significance to them

of even the tiniest functions regained. Thus have the patients inspired the center teams in treatment and rehabilitation activities and in their research to find better methods of management.

Working in this framework, the teams have developed advanced levels of interprofessional understanding and acceptance. The various disciplines in their day-to-day group efforts and relationships in patient care, research or teaching develop awareness of and respect for what each has to offer and understanding of their mutual interdependence. Co-operative relationships develop easily in a permissive environment under good leadership where personal dignity and professional ability are respected. Balanced staff-patient relationships and integration of services have led to unusual accomplishments by the patients. Thus the centers have been able to make an exemplary contribution toward the demonstration of the type of comprehensive care which yields the most successful rehabilitation results.

# Concept of Patient Care in a Respiratory and Rehabilitation Center

DR. JOHN E. AFFELDT

There are 13 respiratory and rehabilitation centers for poliomyelitis in the United States. Because of the strong support and general guidance by the National Foundation for Infantile Paralysis and because of their common goal, their programs are remarkably similar. Each has its own personality, special interests, and talents. In presenting the concept of patient care in a respiratory and rehabilitation center, I speak specifically of the program at Rancho Los Amigos Hospital in Los Angeles, California, but these remarks would apply equally well to any of the other centers.

This center is located in the chronic disease hospital for Los Angeles County. It does not receive acute poliomyelitis patients during the first week of illness but does accept them any time thereafter.

The acute cases are treated in the Communicable Disease Unit of Los Angeles County Hospital. There we maintain a 3-man clinical investigative team to assist Dr. A. C. Bower and his staff in the care and study of the severe respiratory patient. The time between disease onset and transfer to us averages around 2 weeks.

The center has been in operation 5½ years. Up to January 1957, it had discharged 1,590 patients, 56 per cent of whom were in respirators during that admission. At present, direct care is being given to 770 patients, consisting of 140 inpatients, 130 home-care patients (defined as a respiratory patient at home within Los Angeles County under our care), and 500 active outpatients.

The program of the center is basically oriented to patient care and rehabilitation, with 5 objectives:

1. The prevention and treatment of medical complications.
2. The decrease or elimination of respiratory equipment.
3. The restoration of physical activity and function.

4. To return patients to their homes regardless of the severity of their case or the equipment required.

5. The establishment of vocational opportunities when possible.

The hospital program is divided into inpatient, outpatient, and home-care units. The staff for these services is divided into medical, dental, orthopedic, nursing, physical therapy, occupational therapy, orthotic, psychological, vocational, medical social service, electro-mechanical, and school services.

However, the patients are not divided into services; the beds are not segregated; thus all services are equally responsible for all patients. While the patient is undergoing his respiratory weaning program and learning glossopharyngeal breathing, he also is receiving splints and other orthotic devices; mobilization is being achieved by utilizing the standing board, walkers, and the wheel chair; and reconstructive surgery is accomplished or being planned. This requires closely integrated work by all services and minimizes their divisions.

On admission, medical and respiratory evaluation of the patient occurs. Routine pulmonary studies consist of measurement of the vital capacity, alveolar  $\text{CO}_2$  tension (Fig. 41), fluoroscopic study of diaphragmatic motion, clinical evaluation of respiratory muscle strength and patterns of use, and unassisted breathing tolerance. This illustration depicts a nurse on the ward measuring the alveolar  $\text{CO}_2$  with the infrared analyzer to give us some of our respiratory data. The respiratory weaning program is outlined for the patient. This includes the testing and the use of various pieces of respiratory equipment, e.g., cuirass respirators, neck collars, and tracheal or mouth positive pressure apparatus. Glossopharyngeal breathing in traction is started. When abnormal breathing patterns are noted, therapy is directed toward re-establishment of a normal pattern; the respiratory muscles

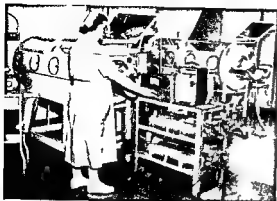


FIG 417 A nurse trained in ventilation studies uses the rapid infrared carbon dioxide analyzer to determine a respirator patient's alveolar  $CO_2$  to be used to guide the respirator pressure and rate settings

are strengthened with resistive exercises mechanical and manual coughing techniques are instituted the tracheostoma is evaluated and closed if such appears indicated the pharyngeal and laryngeal areas are evaluated for function and physical therapy is prescribed for weakness or dysfunction

The orthopedic staff with the assistance of the physical and occupational therapists evaluate trunk and extremity paralysis deformities and potential function Splints and orthotic devices are prescribed while the patient is still using the respirator The standing board is prescribed the nursing service putting the order into effect Sitting and standing balance is worked on along with specific muscle strengthening Substitute functional techniques and devices e.g. balanced feeder or ball bearing arm support are used as muscle strength permits Figure 418 shows the standing board—a respirator patient on it using mouth positive pressure for ventilation In growing children prevention of scoliosis is attempted by body casts and scoliosis already present is treated by straightening procedures including spinal fusion where indicated The patient in Figure 419 is using a metal halo a scaffolding to allow positioning of the spine before and after surgery and tracheal positive pressure for ventilation In such cases the value of combined action by the various professional services has demonstrated itself dramatically The joint action of the medical respiratory and orthopedic staff along with the anesthesiologists has made it possible to do extensive cervical and



FIG 418 A quadriplegic respirator patient with poliomyelitis is placed on a standing board Ventilation is provided by mouth positive pressure using a cuirass respirator pump

thoracic spinal fusions in completely dependent respirator patients as well as other extensive corrective and functional surgical procedures An other aspect of this professional action is the ability to adapt the patient's physical activity program to his respiratory tolerance and to enhance this when necessary with glossopharyngeal breathing or mechanical respiratory aids (Fig 420)

While the physicians are carrying out or guiding those procedures already mentioned related professional services are also working with the patient and his family In Figure 420 the respirator patient is in the pool receiving treatment again being ventilated by mouth positive pressure The psychologists and vocational counselors are exploring the patient's background reaction and adjustment to his situation and his potential vocational opportunities in relation to his present and expected disability Psychiatric consultation is available for the support and guidance of this work The medical social workers explore the family and home situation with the anticipation of mar



FIG 419 A poliomyelitic respirator patient with severe thoracic scoliosis and a fluid neck is placed in a metal frame using the metal halo to fix the head and the neck before and after spinal fusion. Ventilation is provided by positive pressure to the tracheostomy.



FIG 420 A quadriplegic poliomyelitic respirator patient receives physical therapy in the pool. Ventilation is provided by mouth positive pressure using a plastic tube which floats on the surface of the water to deliver the pressure from a pump.

sharing needed community resources as the time for discharge nears.

The weekly patient staff conference is the key to the integration of the program. Here all of the services working with the patient meet (Fig. 421) to discuss with the patient his present status, treatment plan, anticipated goals and time factors.

The Home Care Department staff sits in on this conference participating as needed. It picks up and co-ordinates the complicated problems which go with discharging a patient especially where respiratory equipment is to be sent home. The electricians check the patient's home for safety and the adequacy of all wiring, doors, floors and ramps. A separate automatic power generator is installed for safety against power failure. Attendant housekeeper service is established with training of the families and employees who will be caring for the patient. This department follows and helps the patient at home in routine ways including 24 hour emergency mechanical service (Fig. 422).

An important aspect of care is the planned withdrawal from the hospital for those cases still

requiring respiratory equipment. The visit begins with a few hours only, is gradually lengthened to overnight and then to a week end. Such visitation periods bring to the patient and to his family the realization that even severely involved persons can be outside the confines of the hospital safely and away from the nurses and doctors. This requires careful planning with and training of the family but when the time comes it helps with plans for discharging the patient.

Hospital policy encourages periodic readmission for additional evaluation and rehabilitation. Advances in rehabilitation techniques provide new opportunities for patients once considered static.

In addition to the care program, research and education are two other phases of the work. Clinical research is best done where clinical problems arise and where adequate clinical material is available. The investigative laboratory staff has been placed in the very center of the hospital area. It has ready access to clinical problems and is readily available to the clinicians. The laboratory group studies the different phases of the respiratory system.

The education department provides training for the professional staff as well as for post graduate and undergraduate students. The net result of its work is to improve our own care program.

A subhuman concept of the care program both in our respiratory center and elsewhere is that





FIG 421 A quadriplegic patient drives his electric wheel chair into the staff conference. He uses a mouth stick attached to the controls for piloting the chair. The patient has the opportunity to discuss his therapeutic program with the staff.



FIG 422 The respirator ambulance delivers a patient to her home as a part of the home-care program. The ambulance has its own generator to supply power for the respirator.

procedures developed for severe poliomyelitis cases are useful for patients with other diseases. The respirator may be lifesaving in other diseases. The improved upper extremity orthotic devices for paralyzed poliomyelitic patients are equally useful for arms paralyzed by other diseases.

An example of what the center program can do is best illustrated by a 31 year-old male with onset of bulbospinal paralytic poliomyelitis in 1950. He required a tracheotomy and the tank respirator for care. His vital capacity had risen to 20 per cent of normal where it still remains. He was still using the respirator except for a few hours each day. His extremities were non-functional and he was completely dependent for all of his care. He was discharged to his home with this equipment. This all occurred before the center was established at Rancho.

He remained at home 2 years with no improvement. The home situation deteriorated along with the patient's morale ending up with his return to the hospital in 1953 a bitter depressed man with a divorce pending.

The center program had now been in operation a little over 1 year. The staff began to work with this patient keeping a close liaison and co-operation between the psychologist, medical staff and therapists. His first encouraging improvement was learning glossopharyngeal breathing which increased his time out of the

respirator and allowed him to sit upright in the wheel chair all day. He then learned to use the Robin Aid hook on his right arm and hand using the left shoulder as a source of power. With this he learned to write, drink from a cup, turn pages, light a cigarette and dial a phone. Walking was tried unsuccessfully but he did gain enough leg function to propel the wheel chair slowly. His motivation improved to where he became interested in vocational exploration. Progress was slow but by 1956 he had completed a correspondence course on stocks and securities.

He has now been discharged to our home care program with an attendant to help him using a cuirass respirator only at night. He is employed by a large brokerage firm on a commission basis selling stocks and securities.

This patient represents a before and after situation as regards the respirator-center program. His accomplishments are due to his strong motivation and support from our staff. His motivation is due to staff effort as his morale was very low following his first bitter experience at home. This case best illustrates what an organized staff and program can do toward the salvage of severely involved and incapacitated patients.

**Dr LANDAUER** In this report of the work of physicians with their teams to learn more about what might be done for patients catastrophically disabled from any chronic disease it is important to recognize that the opportunities to do research are at the bedside day by day. Unless society understands and supports the efforts of these teams in a flexible manner so that they take advantage of the opportunities as they arise we will lose much.

Fundamental in our philosophy of grant support to these centers we have built the flexibility to provide for investigation on a permissive opportunistic basis. The budgets are such that they permit investigation of various problems when and as they may arise. In most countries and certainly our own our schools and hospitals are not adequately supplied with unrestricted research funds to be used for what some call scientific fishing expeditions. Our research program in this group of fifteen centers has blossomed I believe through this freedom of operation.

Periodically we bring the teams from the centers together to promote co-operation and collaboration. These conferences of the groups from the centers on patient care and research problems serve many valuable purposes chief of which is the stimulation of further research and progress toward the ultimate goal of more knowledge better patient care and rehabilitation and teaching. The centers have demonstrated the value of this effort so well in their own communities that many of them already are expanding to larger facilities to serve patients with other types of severe chronic disabilities. Administrators of public funds are recognizing now the value of investing money in this trinity of research teaching and care. They are beginning to recognize that venture capital is necessary when we are dealing with such severe problems and trying to expand the dimensions of good medical care.

**Dr. HARTVIKEN** It has been interesting to hear the papers of Dr. Landauer and Dr. Affeldt. It is an imposing task the National Foundation has undertaken in America. I am especially im-

pressed by what Rancho Los Amigos has managed to do for its patients.

We are all convinced of the value of such respiratory and rehabilitation centers as Dr. Landauer and Dr. Affeldt describe with their trinity of care research and teaching. We also surely agree upon the statement that a maximum rehabilitation—a term of Dr. Landauer's—of the severely stricken polio patients is an economic advantage to society as a whole and we know too that through our approach to the polio problem we have learned much about the handling of other chronic diseases.

Our concept of patient care is almost the same as that we have just heard about and so also is our concept of the treatment. We have the disadvantage that in a small unit such as ours we cannot have the highly specialized groups which they have in the centers mentioned. Nor can our experience be of their extent. However we benefit from having the different specialists available at a modern university hospital such as ours. Yet we have the advantage that in such a small unit the staff members get a closer contact with the patients. This creates an atmosphere which in itself is the best factor for motivating the patients and also the staff.

Our program is in short as follows:

1 A respiratory and internal medical program which during the first months will necessarily be the principal one.

2 A general muscular re-education program.

3 A daily living program during which the different forms of technical aids are tried eventually orthopedic surgery is performed and the vocational possibilities are evaluated. In this stage of the treatment the patients return to home is planned. Most of these patients need a new house both in order to rationalize the care at home and to give the patients a chance to move about in the flat and to come out.

The three parts of the program work together most of the time. The stage of the illness and the patients' condition will at any period decide which of them will be the principal one.

During his stay in the hospital the aim is to give the patient a kind of normal life. He shall



FIG 421 A quadriplegic patient drives his electric wheel chair into the staff conference. He uses a mouth stick attached to the controls for piloting the chair. The patient has the opportunity to discuss his therapeutic program with the staff.

procedures developed for severe poliomyelitis cases are useful for patients with other diseases. The respirator may be lifesaving in other diseases. The improved upper extremity orthotic devices for paralyzed poliomyelitic patients are equally useful for arms paralyzed by other diseases.

An example of what the center program can do is best illustrated by a 31 year-old male with onset of bulbo-spinal paralytic poliomyelitis in 1930. He required a tracheotomy and the tank respirator for care. His vital capacity had risen to 70 per cent of normal where it still remains. He was still using the respirator except for a few hours each day. His extremities were non-functional and he was completely dependent for all of his care. He was discharged to his home with this equipment. This all occurred before the center was established at Rancho.

He remained at home 2 years with no improvement. The home situation deteriorated along with the patient's morale, ending up with his return to the hospital in 1953, a bitter depressed man with a divorce pending.

The center program had now been in operation a little over 1 year. The staff began to work with this patient, keeping a close liaison and co-operation between the psychologist, medical staff and therapists. His first encouraging improvement was learning glossopharyngeal breathing, which increased his time out of the



FIG 422 The respirator ambulance delivers a patient to her home as a part of the home-care program. The ambulance has its own generator to supply power for the respirator.

respirator and allowed him to sit upright in the wheel chair all day. He then learned to use the Robin Aid hook on his right arm and hand using the left shoulder as a source of power. With this he learned to write, drink from a cup, turn pages, light a cigarette and dial a phone. Walking was tried unsuccessfully but he did gain enough leg function to propel the wheel chair slowly. His motivation improved to where he became interested in vocational exploration. Progress was slow but by 1956 he had completed a correspondence course on stocks and securities.

He has now been discharged to our home care program with an attendant to help him using a cuirass respirator only at night. He is employed by a large brokerage firm on a commission basis selling stocks and securities.

This patient represents a before and after situation as regards the respirator-center program. His accomplishments are due to his strong motivation and support from our staff. His motivation is due to staff effort as his morale was very low following his first bitter experience at home. This case best illustrates what an organized staff and program can do toward the salvage of severely involved and incapacitated patients.

DR. LANDAUER In this report of the work of physicians with their teams to learn more about what might be done for patients catastrophically disabled from any chronic disease it is important to recognize that the opportunities to do research are at the bedside day by day. Unless society understands and supports the efforts of these teams in a flexible manner so that they take advantage of the opportunities as they arise we will lose much.

Fundamental in our philosophy of grant support to these centers we have built the flexibility to provide for investigation on a permissive opportunistic basis. The budgets are such that they permit investigation of various problems when and as they may arise. In most countries and certainly our own, our schools and hospitals are not adequately supplied with unrestricted research funds to be used for what some call scientific fishing expeditions. Our research program in this group of fifteen centers has blossomed. I believe through this freedom of operation.

Periodically we bring the teams from the centers together to promote co-operation and collaboration. These conferences of the groups from the centers on patient care and research problems serve many valuable purposes chief of which is the stimulation of further research and progress toward the ultimate goal of more knowledge, better patient care and rehabilitation and teaching. The centers have demonstrated the value of this effort so well in their own communities that many of them already are expanding to larger facilities to serve patients with other types of severe chronic disabilities. Administrators of public funds are recognizing now the value of investing money in this trinity of research, teaching and care. They are beginning to recognize that venture capital is necessary when we are dealing with such severe problems and trying to expand the dimensions of good medical care.

DR. HARTVIGSEN It has been interesting to hear the papers of Dr. Landauer and Dr. Affeldt. It is an imposing task the National Foundation has undertaken in America. I am especially im-

pressed by what Rancho Los Amigos has managed to do for its patients.

We are all convinced of the value of such respiratory and rehabilitation centers as Dr. Landauer and Dr. Affeldt describe with their trinity of care, research and teaching. We also surely agree upon the statement that a maximum rehabilitation—a term of Dr. Landauer's—of the severely stricken polio patients is an economic advantage to society as a whole and we know too that through our approach to the polio problem we have learned much about the handling of other chronic diseases.

Our concept of patient care is almost the same as that we have just heard about and so also is our concept of the treatment. We have the disadvantage that in a small unit such as ours we cannot have the highly specialized groups which they have in the centers mentioned. Nor can our experience be of their extent. However we benefit from having the different specialists available at a modern university hospital such as ours. Yet we have the advantage that in such a small unit the staff members get a closer contact with the patients. This creates an atmosphere which in itself is the best factor for motivating the patients and also the staff.

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The three parts of the program work together most of the time. The stage of the illness and the patients' condition will at any period decide which of them will be the principal one.

During his stay in the hospital the aim is to give the patient a kind of normal life. He shall

have a 6-hour treatment in which he is to take an active part. This is his work. In the evening he receives visits or has coffee with the other patients in the living room or attends concerts, shows, films or parties. These events are now arranged by the patients themselves. The social program, except for the personal visits if wanted, should take place outside the ward.

To get the patient and his family acquainted with the new situation, the patient goes home for week ends and holidays as soon as possible. The stay at home is gradually prolonged. Thus we consider an important part of the treatment.

The National Insurance Company pays most of the hospital fees. The rest is paid by cities and counties which also pay for braces and splints. Technical aids and new housings have until now, if necessary, been paid by cities, counties or funds. However, recently the government has suggested guaranteeing a certain amount of these expenses.

When the vocational problems are too difficult to be handled by our unit, we get assistance from the official vocational consultants who are in charge for the disabled in every county. We also have special vocational rehabilitation centers. However, they have not been able to receive the heavily stricken patients, but they help us in organizing homework.

Our worst problem is to get trained or even untrained help in the house for our patients. At our hospital we have a group of nurses who go out by car every morning to give necessary nursing to helpless patients, but they cannot stay for more than 1 or 2 hours by each patient. So I am sorry to say that in some cases the patient becomes a heavy burden on his family.

Dr Landauer has in his paper used the term maximum rehabilitation. What exactly does that mean? It is obvious that the interpretation of this term will reflect upon the entire rehabilitation setup and program. In the American rehabilitation centers, the average duration of

the stay of the patients has been 7 months if I have understood Dr Landauer correctly. With us it might take months to transfer the patients to cuirass respirators and even longer to wean them from artificial breathing, if it is ever possible to do so. The different complications might interfere with any effective physical treatment for long periods. In the meantime the patients are stiffening up and that which remains or is recovered of motor neuron and muscle activity is undergoing an inactivity degeneration. The latent energy and possibilities for active movements can be called upon only through a slow and prolonged process during which the basic strength and tolerance of the patient is built up also. Every new muscular function gives a potential chance for yet further functions. With our new concept of technical aids where even small movements can manage the more complex control systems, activity in practically every muscle might be of benefit to the patient. Our experience says that in a quadriplegic polio patient the rehabilitation takes 3 years and even a longer time. In fact I would keep the patient in the hospital 3 to 6 months after I have seen the last evidence of progress in him.

In order to be able to give all the patients in Norway the same chances of rehabilitation, the Department of Public Health has recently asked one of our staff members to take care of the rehabilitation and home care of all the respiratory patients in Norway.

Our concept of patient care is nearly the same and that of a national program for the heavily stricken patients is also. The differences existing are caused by the differences between a continent like America and our little country as well as by the differences in social legislation.

But our aim is as Dr Landauer predicted: a maximum rehabilitation of the heavily stricken patients. If we can do that, we have built a monument which demonstrates the culture of the nation better than any marble palace.

# Home-Care Programs for the Patient with Respiratory Difficulties

DR JOSEPH BENTON

In medicine as in other branches of social activity advances that are made frequently create new and significant problems. With the development of antibiotics, new surgical techniques and advancement in medical and surgical techniques the life expectancy of people in the United States as well as in other countries throughout the world has significantly increased. This is a wonderful achievement for medical science but has created an entirely new gamut of problems in medicine and in the other professional disciplines.

Recently there has been an increasing awareness of the growing importance of long term chronic illness in an aging population. Suggestions have been made throughout the world in medical literature that patients with long term illness might best be cared for in their own homes on the basis of psychological as well as practical and financial considerations.

I shall discuss briefly some of the factors relating to the origins of home-care programs in general, touching upon basic philosophies and then in detail some of the specific problems relating to the home-care management of patients with respiratory insufficiency.

Organized home medical services have existed in the United States and other countries for a long time. The Boston Dispensary of the New England Medical Center has had such a program since 1790. Similar services were initiated during the 19th century by other voluntary hospitals and independent dispensaries in New York City. Among other countries, Canada, England, France, the Union of South Africa and Denmark have developed home-care programs as extensions of hospital care.

In a recent issue of the *Journal of the American Medical Association*, an excellent survey article describing some 31 organized home-care programs was delineated. This gives an excellent contemporary summary of such programs from administrative, financial, medical, social, co-

professional, vocational and other aspects. Those who are interested specifically in this program would do well to read that article.

While these general home-care programs for other types of patients and for patients with poliomyelitis have evolved along different types of lines, in general the primary concern is with the medical care of patients living in their own home. Other basic needs such as nursing and social services are provided; medications are usually provided also, as well as professional services such as social service, physical therapy and occupational therapy. In addition, housekeeping, services and attendant services are also included. The program usually operates from a central facility—a hospital, a local health department, a medical college, a voluntary health agency or a combination of these. Services are usually limited to indigent and medically indigent groups, although there are some exceptions—for example, programs in Philadelphia and in Montreal where self-supporting patients are served by their private physicians on the traditional fee-for-service basis. Well-organized home-care programs are now integral parts of the contemporary practice of medicine with special significance for the increasingly important long term chronic diseases.

Coming directly to the specific problems relating to patients with respiratory difficulties and home-care programs, the basic premises outlined for home care in the other disease entities represent programs of extension of services for long term patients with respiratory difficulties resulting from poliomyelitis as well as other disease entities. These have evolved as a natural consequence of the overall comprehensive-care mission of the respirator and rehabilitation centers as outlined by Dr. Landauer. In general, these basic principles have been followed but variations necessitated by the unique clinical problems of patients managed in the centers have been introduced.

The long term quadriplegic patient with respiratory insufficiency requiring mechanical respiratory aid poses one of the most difficult rehabilitation problems seen in medical practice. As a corollary to this the philosophy and techniques utilized in such patients can be applied to other long term disabling neuromuscular diseases with or without respiratory involvement.

Probably the most important feature in home care planning is the appreciation on the part of the patient, the patient's family, the professional staff and the community that a natural course leading to eventual home discharge is part of the overall scheme for such patients unless specific contraindications exist. This premise is of primary importance. Many patients find it easy to become psychologically deconditioned to the thought of returning home since the respirator and rehabilitation center programs have been developed to such a degree that many of their medical as well as nonmedical needs have been adequately subserved during hospitalization.

Because of the multitude of problems confronting such patients, predischARGE planning for home care begins early in the course of the patient's hospitalization. Home-care plans for the patient with respiratory insufficiency in general differ from home care for patients with other long term illnesses in that more supervision directly or indirectly is required with expedited readmission to a center in case of a medical emergency. This point was touched upon by Dr. Afseldt. This requires a much more integrated relationship to home care on the part of the professional staff of the center. However, there are some patients who, because of complete family dissolution due to the catastrophic nature of the disease process, will not be able to be returned home. These will require indefinite center care, not primarily for medical reasons but for social ones.

In the predischARGE planning for home care, center and home-care personnel in joint conferences carefully consider the following specific points:

1 *Medical Indications or Contraindications* Where the medical and surgical status is unstable or if the patient requires medical gases such as oxygen, home care is contraindicated. Aside from these, there are no medical reasons why patients cannot be placed in a home-care situation. Most patients can return home with

respiratory equipment including the full body respirator where this is required. In some centers, full legal responsibility is assumed for the patient where the program is an extension of center care.

2 *Equipment Recommendations* All equipment, respiratory as well as other varieties, must be on medical prescription. Long before home care discharge planning, the patient has been studied intensively and his ventilatory requirements are known. Consideration for (a) the ventilation and safety factors for the patient, (b) the fit and preference of type of equipment, particularly for cuirass or shell respirator, and (c) mobility factors are carefully evaluated. It has been a custom in some centers if a patient does not have at least 2 hours of unassisted tolerance in breathing, excluding glossopharyngeal breathing, to insist upon home installation of a source of emergency power supply.

In addition to the respiratory equipment, when indicated in severely disabled patients, a variety of assistive and self-help devices—hydraulic lifts, prostheses, splints, frames, slings, wheel chair and attachments, mouthsticks, reading and electronic devices—are also projected as required. Of great importance is the indoctrination of the patient's family and attendant. This is actually done in the center while the patient is undergoing treatment. Equipment maintenance and operation, nursing, physical and occupational therapy care, and other aspects of patient care are demonstrated to responsible members of the family. In addition, instilling confidence in the family is valuable in that the family is made to feel a member of the therapeutic team. One center has a regular system of equipment maintenance plan staffed by center personnel.

3 *Personnel in the Home* I shall not enter into detail in this except to say that where necessary, professional personnel, not necessarily a registered nurse, is made available to help in the home situation. These individuals, where families cannot afford the cost, are subsidized by payment to the family by the National Foundation for Infantile Paralysis.

4 *Vocational Aspects* Patients in centers demonstrate a wide range of readiness for vocational counseling. If this is manifested, the counsellor sees the patient after he has the established familiar hospital routine and is not acutely ill. Testing procedures and interviews

are integrated with nursing and therapy programs and in keeping with the patient's physical capacity. The educational process in children or in young adults who might require this is maintained by virtue of established community agencies such as Boards of Education under the special education program which many cities in the United States maintain.

**5 Maintenance of Patient at Home** In addition to the regular medical and nursing care the following prescribed physical and occupational therapy programs may be instituted: (a) maintenance of range of motion exercises (b) sitting a minimum of 1 hour a day in a wheel chair (c) re-education and strengthening exercises (d) standing for 30 minutes to 1 hour where feasible (e) the continued use of prescribed assistive equipment and (f) activities of daily living (ADL) training including housework simplification for housewives. In some centers an attempt is made to readmit the patient for short stays at intervals of 3 to 6 months on an elective basis for re-evaluation of ventilation status and cardiovascular pulmonary renal skeletal-motor skin and other system review. Indicated laboratory studies are repeated and the patient is re-surveyed to determine his change in status.

The mortality rate of this group ranged between 4 per cent and 6 per cent in the 4 year period. About half of these deaths might have been prevented by earlier respirator placement when the patient developed respiratory infection.

The costs for home care per patient average about \$300 to \$500 per month depending upon the geographic location in the United States. In some areas patients' families contribute to these costs in accordance with their ability to pay; in others Federal State and local governing bodies assume varying degrees of financial responsibility.

Finally the results of such home care programs in one center (a series of 90 patients) were re-evaluated in terms of function 1 year after discharge to home care on a maintenance program. Of these 90 at the end of the year of home-care management 88 were sitting in wheel chairs daily 75 were standing regularly and 42 were walking at various levels of proficiency 79 had partial or complete independence in feeding and writing activities and 53 had partial or complete independence in hygiene and dressing.

In conclusion outstanding isolated examples

of physician lawyer engineer student and houses the patients exist who have been returned to productive existences. This aspect holds the greatest challenge for the total-care programs required for these severely disabled patients. However it should be emphasized that return to a job is not necessarily the sine qua non of a rehabilitation program since in many patients retraining to an existence of self-care and sufficiency in daily living also must be considered a notable rehabilitation achievement. Such individuals can then live in human dignity and be independent for performance of the basic requisites of self feeding, dressing and toileting.

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Dr SHEPHERD Dr Benton's fine paper on "Home Care Programs For The Patient With Respiratory Difficulties" was sent to me in Buenos Aires for study. I found the first part of his article profoundly depressing.

Here we were in Argentina swamped with an aftermath of postepidemic patients just pulling out of a 10 year dictatorship that had disorganized the country with no home-care laws for the crippled that could be discovered (though we searched through the library of Congress and inquired at all the different Departments of Public Health that we could think of) and without funds specifically allocated for either home respiratory equipment or attendants and apparently from Dr Benton's article the secret of the amazing results obtained in rehabilitation in the States was the existence of all-embracing programs and unlimited funds. These programs went way back to the 1790's. I quote: "The Boston Dispensary of the New England Medical Center has had such a program since 1796."

Funds were apparently completely adequate since State and Federal grants provided money for the totally and permanently disabled. The National Foundation paid all the attendants needed.

As I said the first reaction to Dr Benton's paper was one of depression but on rereading his article and on studying his analysis of the complicated setup necessary for the rehabilitation of these terribly handicapped individuals the same pattern began to show in print that I had seen 5 months before on visiting the great respiratory centers in the United States and it certainly was not a picture of coldly planned programs nor of millions constantly poured out but rather the image of an amazing group of people with an extraordinary attitude and I say extraordinary advisedly.

During last September while visiting the States I was impressed by this attitude of the respiratory team. They did not seem to realize that the patients they were treating were quadriplegics with respiratory difficulty.

The whole time I was there I didn't once hear

the *Vale la pena?* Para que? No hay nada que hacer? (Is it worthwhile? What for? They haven't a chance) that we had had thrown at us from the beginning of the epidemic even from the lips of those who should have known better. The thought didn't even seem to occur to them. On the contrary their immediate reaction was "Well now what has to be done to get this patient back into meaningful living."

I was introduced to completely disabled persons who were earning their own living at home. I was referred to colleagues who were back at work in chest respirators and I met others who were gainfully employed though still using glossopharyngeal breathing.

On inquiry I found that apparently not all patients were able to return to full employment but the interesting thing was that the group seemed to accept the second best only after a long dedicated trial of every single possibility.

Every nation has its outstanding individuals whose elongated shadows as someone has said be come living institutions but here were many groups with the same enthusiastic optimistic bracing attitude running through every member of the team.

The success of a rehabilitation program certainly demands the existence of a good program but the secret is not in the program but in the obtaining of a team formed by a staff of high quality and high motivation.

The persons affected by polio are exceedingly difficult and trying patients and it is all too easy to pass on the responsibility of their care to someone else. At these centers I found that the team as a whole shared the responsibility of the future welfare of the patient and that each member was ready to think constructively and originally within the overall plan. Sometimes this was even put on one side if it was felt that it was not to the fullest benefit for the patient.

At one fine respiratory center for instance I asked why the State Vocational Department was not used. The reply was the not polished but certainly graphic "It stinks!" I don't exactly remember what the difficulty was but it had

something to do with the retraining of a Ph D to cobble shoes. Well, what do you do? We go out and sell the patient ourselves was the answer.

With that warm relationship toward the patient pervading the whole group, there are bound to be results.

The epidemic that hit Argentina last year with an incidence of over 6 000 paralytic cases (in a population of 18 000 000 inhabitants) brought a terribly high mortality rate during the first 3 months. Seventy-four per cent of the cases were under 5 years old and 22 per cent in the Buenos Aires area were respiratory forms.

Though we had been through other polio epidemics (in 1933, 1942 and 1935) the number of respiratory cases never had been so appallingly high. We found ourselves completely unprepared to tackle the problem. The medical group in general was out of touch with the latest developments; the hospitals were inadequately staffed and badly run; the co-professional services were negligible and respiratory equipment was completely insufficient.

We were swamped by the severity of the attack. Every other child seemed to have breathing difficulties and since in our hospital alone 30 to 40 cases (paralytic) were coming in per day the situation was desperate. We had started to tracheotomize and hand breathe the children when the wonderful gift of iron lungs from Uruguay and the States reached us.

*But not only equipment came.*

We never can be sufficiently grateful for the help we received from our colleagues from other countries. We were overwhelmed by the generosity of those who contributed and by those who came.

It was the first time that we had come into the orbit of the help offered by the world medical family and it was a most moving and enriching experience.

It was an extraordinary privilege to have the direct advice of consultants from other countries for our own Lauristas, Miguelitos and Moniques.

The experience so gained is what has helped me feel up to dare to think that in a year we can catch up the lost 10 years when we were isolated from all new rehabilitation techniques.

However, the privilege of having foreign experts helping us led to slight complications and the need for some careful steering when the

consultants who visited us in the morning counseled tracheotomy and P.T. as the only solution while those who came in the afternoon believed only in rocking beds and cuirass respirators! Many of our patients bear the scars of the two opinions on their necks and on their espaldas (anterior-superiores). But they are alive and being rehabilitated.

Home-care plans for the patient with respiratory insufficiency demand as Dr. Benton says more supervision directly or indirectly than with other long-term illnesses and an expedited readmission to a center in case of medical emergency. This requires (I quote) a much more integrated relationship to home care on the part of the center's professional staff.

Adequate home care of the respiratory patient is based on the existence of a well-organized respiratory center and the creation of such a center is the first step in any home-care program.

We had to start from scratch and from data carefully culled from the visiting consultants, a plan for a modern respiratory center was presented to the authorities. Since we now have a Ministry of Public Health interested and keen on rehabilitation, the program was accepted and our first respiratory center was opened in Buenos Aires last September with a capacity for the treatment of 30 to 40 cases.

These last few months have seen the knitting together of the members of the team and the working out of an adequate balance between research and rehabilitation. (We still have not completely solved the problem created by the Rehab Groups' habit of disappearing under cover of darkness when the Whittenberger-Ferris film is being expounded or re-expounded.)

The single most effective measure in focalizing this objective has been the organization of the Occupational Therapy Department under specialized technicians lent by the British Government and by the Rancho Los Amigos Respiratory Center and through the financial aid of the Argentine Polio Foundation (ALPI). We are hoping that the future will see the early setting up of a program for the preparation of specialists in this aspect of rehabilitation.

Our psychiatrists have been of amazing help during the epidemic and postepidemic phase and we have learned to depend on them for a

practical solution not only of the patient's problems but also for those that appear through the inter-personnel relationships during a post epidemic

In the past we have had some wonderful cases of total rehabilitation of the severely disabled but these have been isolated incidences and have depended upon the efforts of a distinguished and dedicated physician. In the rest

of the cases we have been satisfied with survival.

But survival is not the answer and cannot satisfy us now that we have seen the results of an integrated program of rehabilitation. The incredible power of the words we have seen lead us to hope that our patients too will be given the chance of seeing transformed into reality the things that up to now have only been hoped for.

## *Vocational Aspects in the Rehabilitation of the Poliomyelitis Respirator Patient*

MISS RUTH LOCHER

The major concern in the early phase of poliomyelitis is survival. Even in the early postacute stage the patient is exceedingly anxious about his chances to live. Gradually other ideas begin to become conscious fleetingly at first but not long enough to add more worries to his collection of fears and acute anxieties. These ideas center on the questions of "What shall I be like? What can I do? Can I walk again?" If the patient is an adult man one can expect rather soon some expression often indirect of concern about his job or his work. We anticipate this thought and try to help the patient talk about it. Early in the treatment process the only assurance that can be given is that the matter of work, employment or income producing activity will be considered and that every effort will be made to help him become self supporting. The staff of the treatment center should recognize that the meaning of work is greater than just a source of income. When we are truly aware that being productive has emotional and social as well as economic significance the patient senses the reality of the assurance that has been given him.

Along with the many worries the patient has about his condition and the future he also has the immediate problem of the cost of hospitalization and medical care. The family too is confronted with this problem and with the loss of current income the situation could be so serious as to interfere with successful medical care. We need to acknowledge that much anxiety is eliminated at the beginning when the patient and his family learn that the National Foundation for Infantile Paralysis will give whatever help is needed and as long as it is needed to provide the best medical care and rehabilitation that is available. We know that even small children can worry about the cost of medical care and it can interfere greatly with the treatment program. One 8 year-old girl suffered severe stomach upsets and headaches

weeks after the acute stage. No organic basis could be found for it. The trouble cleared up when she revealed worries about the cost of care and the fear that her father would need to sell his car—and could not visit her—and his house so that there would be no place to live when she left the hospital. When I told her that the National Foundation for Infantile Paralysis would help her Daddy and that there was no need to sell anything she sighed so deeply with relief that the patients in adjoining beds could hear her! With one major worry eliminated the total treatment program has more chance of success.

While the patient is experiencing an overwhelming alteration in his body and undergoing strange new procedures in his care others nearby also are having a miserable time. No individual lives in a vacuum fortunately and all of us have families with whom we share our lives. We need to remember the families as they play a vital part in the rehabilitation of the patient. The influence of the family may be the deciding factor in achieving a vocational goal. Therefore it is necessary at the beginning of the illness for someone to help the family with the worries that confront them. Mothers, fathers, wives, husbands, children too—all suffer deeply. Once the crisis is past and life is saved the family begins to worry about the ultimate outcome of the disease. Future plans are disrupted and uncertain. Although no immediate solution is evident at least these concerns need to be recognized and talked about. At the same time current problems of living come into view. Daily affairs are ignored or shoved into the back ground with the onset of the disease and continue to be handled on an emergency basis until danger is past. Then the reality of responsibilities and obligations hits with full force. Fear about the present as well as the future creates additional burdens.

The medical social worker meets the family

■ soon as possible after the patient is admitted to the hospital. This is the beginning of a contact that continues throughout the period of medical care and often after the patient is discharged to his home and community. From the start the medical social worker helps the family through the weeks of worry and strain that follow. She is prepared to assist them in securing information and help from the doctors and to interpret further to them the various aspects of medical care. She also directs them if necessary to the various local resources—social, economic, religious or educational. If the reactions to the current situation are extreme, the medical social worker in collaboration with the medical staff will attempt to secure psychiatric help for the family.

During this period one learns from the family many things about the patient: what kind of a person he is, what his particular role is in his family with whom he has the closest relationship. Later more is learned about the patient particularly in connection with his past activities at school, at work, at home and in the community. We find it important to know how he has faced illness, frustrations and achievements in the past. All this has real meaning in helping the patient to return to a useful life after the treatment program is completed.

Although knowledge of the patient is essential, it is equally important to understand the family. Their ability to face this overwhelming situation to make adjustments in their lives and to utilize to advantage whatever resources are made available to them will give evidence of the support they will give to the patient when he needs it. The staff in the hospital can have a powerful ally in the family group, or it is possible that our knowledge of the family will reveal the fact that no help will be forthcoming. Under such circumstances total rehabilitation is more difficult and it is better to be cognizant of the situation early.

Throughout the convalescent period and the rehabilitation period it is necessary for the medical social worker to establish then maintain a good relationship with the patient. As his condition improves, some of his attention is directed toward the functional use of his body rather than entirely upon the devastating physical condition. Some of his fear, anger and depression is lessened. This change may be slow

and while the patient is struggling toward an adjustment, the medical social worker needs to share thoughtfully with the rest of the staff the knowledge she attains about the patient's attitudes. At the same time all the staff working with the patient must keep each other informed of their observations of the patient's reactions. Thus it will be possible to adjust the treatment program to the patient's particular needs. He will have an opportunity to try out some of his ideas to test his abilities and to direct his energies toward a practical use. The patient will then become an active member of the treatment plan.

Motivation toward the use of one's capabilities is the sine qua non of rehabilitation. Where none exists it is doubtful that even the most expert staff with the best psychiatric consultation can create it. Fortunately most people have a spark and this can be nurtured and stimulated by the rehabilitation group which truly understands the patient, his family and the community from which he comes. Careful evaluation of the patient's motivation must be made early. When help is needed to promote the patient's drive, this help must be constant and a part of the treatment from the very beginning.

While the patient is in the hospital, major focus is upon him and his progress. The family too receives attention and as a consequence gives much help to those providing care for the patient. However, as the patient moves toward fuller rehabilitation, an intimate knowledge of his home community becomes increasingly important. Not only will the home-care program depend upon community support and understanding but vocational plans need to be verified by the community. Work that may seem practical and feasible from a medical viewpoint—that is to the hospital staff and the patient—may be entirely unrealistic in the particular geographic area in which the patient will live. Motivating the patient to achieve something that is neither practical nor possible when he goes home leads only to disaster.

For the sake of economy of energy, time and money as well as of patient and family morale, the medical social worker should become acquainted with the community. This means that she must have firsthand knowledge of the home situation, the cultural, social, economic, etc.,

tional and employment resources of the community and last but not least the attitudes toward disabled individuals. Such information cannot be secured adequately by correspondence or by studying statistics about the area.

Let me illustrate briefly why the hospital through the medical social worker has responsibility to understand the community. An adolescent from a sparsely populated area was near the end of his treatment program and we planned to send him home. Many things needed to be considered. Continuing medical care facilities for emergency hospitalization, schooling, periodic nursing supervision and completion of the vocational plan had to be arranged. The family attempted to interpret these needs in their community but met with little success. When the medical social worker arranged to spend several days in this small city she learned quickly that the community generally was horrified that the hospital considered sending a young man home when he could not breathe without mechanical assistance and was totally dependent upon others to meet his personal needs. First the local doctor had not had experience in caring for such a patient. Next the community hospital flatly refused to admit such a patient or even to have a tank respirator in the building. The school superintendent was irritated that the hospital would so much as think of risking this young man's life by sending him home. The public health nurse refused to take any responsibility for supervising home care. However after 2 days of interpretation and discussion the community became most interested and co-operative. When the young man's condition was really understood everyone was eager to help him again become a useful citizen and they did so.

This same kind of interpretation and preparation is needed no matter where the patient lives. It is important to have someone from the hospital who knows the patient and is familiar with his present condition and future outlook discuss these things fully with those who will have close contact with the patient after he returns home. Employers too need help in comprehending the patient's situation. Their first reaction upon seeing the patient is one of shock and frank disbelief that anyone so severely disabled is capable of working in any capacity. Along with the shock there is considerable fear

too. Explanation of the patient's total situation usually stimulates a willingness to give the patient a chance to prove his abilities.

Two other factors are extremely important in reaching a vocational goal. First the kind of work the patient does must be of his own choosing. A job for the job's sake is not held long. Second the job should not be created solely to give the patient something to do. "Made work" is not satisfying and even though the remuneration is adequate the sense of accomplishment is lacking. The resultant feelings of dissatisfaction and discouragement can materially delay or defeat the achievement of real vocational goals and seriously affect the patient's total social adjustment.

The patient needs consistent help in thinking positively of future vocational goals. Whatever ideas occur to him must be considered seriously and explored. Day by day, week by week emphasis upon the practical aspects of activity is related to the theme of vocational planning. Since our cultural pattern includes economic independence and support of the family motivation toward this end is sponsored by careful and continual discussion of functional abilities and intellectual interests. Surprising suggestions can come from patients in these circumstances. For example a young man who had been a dairy farmer following 4 years service in the Army finally asked if he could try to operate a sewing machine. Had the staff not known or understood this man one look at his frame (6 feet 2 inches high) would have created dismay and aroused question about his personality. We could have scoffed at his idea or suggested courses in bookkeeping and accounting or have delayed making changes in the treatment program that would lead directly to his desired activity. However as the situation developed efforts of the total staff were directed toward the goal that was requested. Wholehearted support and encouragement by a staff that had been kept informed of the man's emotional as well as physical progress led to a new vocation that was creative and financially rewarding. Commercial embroidering of athletic and sports wear was a service that proved to be needed by various groups in the community. In addition the relationship between this man and his wife was sound and she gave acceptance

and encouragement to the extent that he could feel that he had resumed a masculine role as well as financial support of his family.

Planning and developing a realistic and satisfying vocation with the patient requires a thorough understanding of the patient, his family and community. Selection of employment possibilities cannot be made solely on the basis of intelligence and aptitude tests, physical ability or disability. Training a man for a job only because he has certain muscles and specific intellectual accomplishments can be a total waste of time. Focus on the patient as a person is paramount for success. Furthermore, the staff members of the rehabilitation unit need to have a common philosophy and the same kind of in-

centive to help the patient. They must speak the same language and understand each other in order to share fully all essential information that relates to the patient's program. It is important too that each member of the staff recognize that everyone has something to contribute in this whole program. Mutual respect for different skills in the treatment team is as important as having respect for the individual differences in the patients. Interdependence of the members of the hospital staff upon each other is as great as that of the patient, his family and community. When all work together, rehabilitation can be complete and the patient can be expected to return to a useful and productive life.



## DISCUSSION

DR PEJME The indications for respirator treatment vary at different clinics. It follows that the results as to rehabilitation also vary for with broader indications for this treatment the occupational rehabilitation improves.

In Stockholm we apply stringent indications for respirator treatment and therefore have rather low figures for occupational rehabilitation. In this connection we cite our cases with respiratory insufficiency from the epidemic of 1953. The results with regard to occupational rehabilitation were good in the nonrespirator group but entirely different in the other.

The total number of patients with respiratory insufficiency amounted to 144. 89 of them were treated in respirators and 55 were not considered to require that treatment (Table 182).

TABLE 182 PATIENTS WITH RESPIRATORY INSUFFICIENCY BY GROUPS

<u>Nonrespirator group</u>	55
After 3 1/2 years	
Fit	18 = 33%
Partially fit for work	37 = 67%
Total disability or death	0
<u>Respirator group</u>	89
After 3 1/2 years	
Fit	2
Paralysis but fully fit for work	15
Partially fit for work	17
Under rehabilitation	4
In respirator	13
Requires constant attention but not respirator	6
Deaths	32

Patients with respiratory paralysis who undergo vocational training may be classified in three groups:

1 Those in whom a satisfactory respiratory function has been restored after grave paralysis.

2 Those whose respiratory function is still so impaired that artificial respiration has to be applied for part of each day.

3 Those who still have severe respiratory insufficiency.

In each group there is the additional problem of the extent of paralysis in other parts of the body. Patients with respiratory insufficiency whether of bulbar or spinal origin often have paralysis of the upper extremities too. The difficulties of rehabilitation are especially great in these cases and undoubtedly present today our biggest problem in this particular field. In those with paralysis of the lower extremities the problem is much simpler for most of them are quite capable of doing useful work from a wheel chair. Here the problem is largely one of transport and usually can be solved nowadays with invalid carriages and appropriate technical measures in the home and at the place of work.

The paralysis of the trunk itself generally can be overcome. In milder cases the patient masters this problem himself; in severer cases supporting appliances are required. Here a spring reinforced cloth corset generally suffices; only occasionally is it necessary to apply a leather corset reinforced with metal bars. In rare instances the patient has thoracic paralysis of such type and degree that the spring reinforced corset impedes his breathing. If in such cases the rest of the trunk has not been severely paralyzed we have overcome this problem by shortening the corset somewhat. If this is out of the question the problem will be more delicate and the possibilities of working in an upright or sitting position will be reduced. However in our experience a situation of this kind seldom arises. In Sweden we are now trying out a plastic chair fitting with a bowl shaped support for the thorax; perhaps it may be of benefit to these patients who are unable to wear a corset.

There is no need to consider here the first category of patients—those who later have a fully satisfactory respiratory function—for any other paralysis that may persist will place them in a group where the rehabilitation problems are purely general and not the specific ones we are concerned with here. However I shall mention a few cases exemplifying the excellent working performances that have been achieved later on despite severe poliomyelitis in the acute stage (Table 183).

TABLE 183 GAINS MADE BY PATIENTS WITH SEVERE POLIOMYELITIS

SEX	AGE	DISEASES	TRACHEOTOMY INDICATION	RESPIRATOR TREATMENT	LOWEST VITAL CAPACITY	SUBSEQUENT HIGHEST VITAL CAPACITY	OCCUPATION ETC
♂	24	Polio with paresis bulbar and spinal Bronchopneumonia with atelectasis Myocarditis acute	Palatal paralysis encephalitis	Engström 1 1/2 days	1 750 cc	2 900 cc (Sept 1953)	Engineer
♂	3	Polio with paresis bulbar and spinal	General paralysis encephalitis	Engström 15 weeks	400 cc	2 400 cc (Feb 1955)	Classical
♀	25	Polio with paresis bulbar and spinal	Pharyngeal paralysis	Engström 10 weeks	Indeter- minable	1 900 cc (July 1954)	Housewife (Childbirth 1946)

The situation is different with the other two groups. The most important of these in practice is Group 2 where in spite of everything, rehabilitation often can and should be carried through. In our experience rehabilitation of patients with diaphragmatic paralysis is well worth while.

Diaphragmatic paralysis is virtually never total at this stage yet it may be serious. However the combined effect of the restored thoracic and diaphragmatic breathing usually suffices for the essential respiratory movements during the day though in some cases it may be otherwise nocturnally. Patients of this type are found to make up the bulk of Group 2 for while fully conscious by day they have control of their respiratory function but when asleep their breathing is insufficient and the exchange of gases inadequate. The expiration is usually affected most with greatly elevated CO<sub>2</sub> values not until later does the O<sub>2</sub> saturation fall according to our experience in Stockholm. This state of affairs is especially evident after a prolonged observation period. At the Contagious Diseases Hospital in Stockholm we have treated

a number of these patients in whom we observed gradually developing fatigue going over into short periods of semistupor. Here arterial puncture has disclosed acidoses and elevated CO<sub>2</sub> tension as well as reduced O<sub>2</sub> saturation. Respirator treatment with a cuirass or rocking bed has brought about a speedy improvement in this respect even when resorted to only at night.

Table 184 shows a woman who had the acute stage of polio in 1953. Three years later we found her fatigued and arterial blood puncture showed pH = 7.6 CO<sub>2</sub> tension 6 mm Hg. After about 4 months in a rocking bed nocturnally the values were normalized or almost normalized. At the bottom are the normal values from our laboratory.

One male patient (Table 185) has nevertheless shown persistently elevated CO<sub>2</sub> levels in spite of these measures but after regular nocturnal treatment in a rocking bed his mental alertness at least has been normalized. We have transferred him to a convalescent home where he makes small articles from a wheel chair by day and sleeps in a rocking bed at night. He spends

TABLE 184 GAINS MADE BY WOMAN PATIENT

SEX	BORN	POLIO	TOTAL PLASMA CO <sub>2</sub>	BLOOD pH	CO <sub>2</sub> TENSION	O <sub>2</sub> SATURATION	VITAL CAPACITY
♀	1931	1953	Aug /56 64.5 vol % (29 mMol/l)	7.26	67 mm Hg	84%	1956 500 cc
			Dec /56 49.5 vol % (22.3 mMol/l)	7.41	34 mm Hg	89%	
	Normal		50-65 vol % (22-30 mMol/l)	7.35-7.45	30-43 mm Hg	94%	

TABLE 185 GAINS MADE BY MALE PATIENT

SEX	BORN	POLIO	TOTAL PLASMA CO <sub>2</sub>	BLOOD PH	CO <sub>2</sub> TENSION	O <sub>2</sub> SATURATION	VITAL CAPACITY
♂	1927	1953	Sept /56 87.3 vol % (40 mMol/lit)	7.20	99 mm Hg	74%	Acute 300 cc
			Jan /57 86.1 vol % (38.7 mMol/lit)	7.21	68 mm Hg	95%	Feb /55 1200 cc
	Normal		50-65 vol % (22-30 mMol/lit)	7.35-7.45	35-43 mm Hg	94%	June/55 900 cc Sept /56 900 cc

week ends in his own home without the help of a respirator

As regards Group 3 patients with severe respiratory insufficiency we have in Sweden little experience of a restored capacity for work. Theoretically patients who are able to use their arms and hands satisfactorily might well do manual work even if they need respirator aid or breathing but experience shows that such work is often made impossible in these cases by

severe paralysis of the extremities. In Stockholm at least we have no patient of this type working largely outside the field of purely occupational therapy. However we do have 1 or 2 patients who have been discharged home under permanent intratracheal treatment with a positive pressure respirator and have had to be content with this since they are so paralyzed in other parts that vocational work is out of the question.

# Final General Session

FRIDAY AFTERNOON, JULY 12, 1957

(This Session Convened in the Salle du Conseil General)

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## *Chairman*

DR ROBERT DEBRE

Faculty of Medicine

University of Paris

Paris

## *Speakers*

DR C H ANDREWES

National Institute for Medical Research

London

DR SVEND CLEMMESSEN

Kommunehospitalet

Copenhagen

MR BASIL O'CONNOR

International Poliomyelitis Congress

New York

## Summary on Viruses

DR C H ANDREWES

It would be impossible in 15 minutes to summarize adequately over 30 papers on viruses to say nothing of the contributions to the discussions. It would be especially hard to deal with the excellent papers on tissue culture and on general virology which form such a useful background to the presentations on more specialized themes. Therefore I propose to confine my summary to two matters which have been in the forefront of our discussions—the position of the ECHO and Coxsackie viruses in relation to poliomyelitis and properly and inevitably the present status of vaccination.

### THE ENTERIC VIRUSES

After what we have heard we are beginning to see as a whole a family of viruses which is characterized by a habitat in the intestinal tract of mammals, very small size, spherical shape, resistance to ether and perhaps a definite range of stability in varying pH's. Certainly some other viruses such as adenoviruses can be found in feces but it should not be difficult to exclude these from the family of enteric viruses which includes the poliomyelitis, Coxsackie and ECHO viruses together with others inhabiting the intestinal tracts of monkeys, swine, cattle and mice. We have learned of and I have already mentioned some properties which they all share. They differ as seems to be emerging in varying ability to develop certain potentialities—pathogenicity for the primate nervous system, also for suckling mice and again for primate tissue cultures. There are thus defined three groups fairly distinct yet with undoubted overlaps in their properties.

The Coxsackie viruses as Dalldorf has described have pre-eminently an ability to infect suckling mice, the mode of attack being rather different for the A's and the B's. The A's—some of them—will grow in and destroy HeLa cells but not monkey kidney cells. Still fewer will grow in eggs or affect the central nervous system of primates. Coxsackie A 14 however seems particularly apt to do the latter and the

Russian polio type IV, which is much the same as Coxsackie A 7 seems to cause a disease like paralytic polio in man. Dalldorf suggests that other Coxsackie viruses isolated from stools of polio cases should not be too readily laughed off. The Coxsackie B's are a little nearer to ECHO viruses in growing as a rule readily on monkey kidney as well as HeLa cultures. Several types of disease in man have been described as being caused by Coxsackie viruses. Gear has discussed myocarditis in this connection and Furcolow aseptic meningitis due to Coxsackie B 5.

This brings us close to ECHO viruses of which Wenner, Melnick and Karzon have described several as causing aseptic meningitis. These resemble polio viruses in pathogenicity for primate tissue cultures but not as a rule suckling mice; they differ from polio in their much smaller tendency to cause nervous system damage in primates. There are some awkward ones particularly the European Type 9 ECHO virus which is antigenically like classical ECHO 9's but is also related to Coxsackie A 9 and puts down suckling mice. Perhaps it will be wise to transfer this to the Coxsackie group yet others remain whose position is still obscure. If the O of ECHO stands for Orphan because of absence of association with disease it looks as if fewer and fewer of the ECHO viruses still qualify for continued residence in an orphanage.

The properties of typical polio viruses we all know. But there are strains which have lost pathogenicity for primate nervous system and even for primate tissue cultures and some which are pathogenic for baby mice though not as a rule causing myositis like the Coxsackie A's. I imagine one could present many a virologist with a modified virus and forbidding the use of serologic tests, defy him to say whether such a virus should be called polio, Coxsackie or ECHO. Melnick has suggested that all these viruses might have had a common evolution. I would go further and suggest that they are of one rather close family and may have become

separate only lately. Perhaps they may be likened to a mountain range with—from the point of view of what we may perhaps call the nervous primate—three striking peaks: polio viruses 1, 2 and 3. Possibilities of recombinations among them have been suggested apart from such a possibility. I feel that serious and persistent attempts to suppress some of their potentialities and extol others might lead to surprising results and convince all that these enteric viruses form a definite family.

### EPIDEMIOLOGY

We are getting accustomed to the idea that with improving hygiene we are in danger of having to pay the price for less typhoid and dysentery by suffering more paralytic poliomyelitis in older persons. What is true of polio seems likely to be true also of infections by other enteric viruses. The recent widespread European outbreak of ECHO virus 9—meningitis—warns us of this danger. Virus meningitis—Bornholm disease and other Coxsackie infections—may really be like paralytic polio up-and-coming relatively new diseases of civilized communities. By the next Congress we may be talking much more seriously of vaccines against these other virus infections.

### POLIOMELITIS VACCINES

There has indeed been a change in prospects for preventing polio since the Third Congress 3 years ago. The Salk vaccine was then just beginning to be used on a fairly large scale and we knew very little about its efficacy. Now we know from experience during the past 2 seasons that it has proved capable of preventing a lot of paralysis in children. That is something of which the Foundation and its workers can well be proud. The vaccine does not prevent every vaccinated child from getting polio but the incidence which is 100 per cent perfect exists only in our dreams. Looking back still further to the Second Congress in Copenhagen who could have believed that the tissue-culture work reported there by Dr John Enders would have yielded such amazing results in 6 years not only as regards polio vaccination but in the field of virology generally? I am sure we may expect further exciting discoveries to follow from the new work on tissue culture of which we heard on Wednesday.

There are those who believe that a killed vaccine which can be made perfectly safe can achieve all we need in the prevention of paralytic polio. Others point to the theoretical advantages—and they are many—of an attenuated virus given by mouth. If a course of Salk vaccine will protect children throughout the most vulnerable period of their lives that is surely good enough. Salk has at this meeting adduced forceful arguments based partly on theory and partly on experiment why such a course should be adequate. He maintains that even if antibodies fall off as nearly all antibodies do and even reach a nondemonstrable level yet the tissues remain sensitized able to respond immediately to fresh contact with virus and prevent not intestinal infection but paralytic complications. Others doubt some of these arguments and suggest that there is an element of wishful thinking about them and point out that immunization with other killed viruses does not in fact lead to an adequately persisting immunity. Drs Koprowski and Sahni expect an attenuated vaccine to give more durable immunity with greater freedom from a number of disadvantages. Against this there is increasing evidence from several quarters that the attenuated variants are less stable than was once hoped and that they may revert to greater virulence in the intestinal tracts of inoculees and still more in the intestines of contacts of vaccinees. The proponents of an attenuated vaccine point out that in some less developed countries all children are exposed and develop antibodies to all 3 types early in childhood. The danger of reversion to greater virulence is unimportant there since the environment is saturated with virulent viruses anyway. In just such areas economic circumstances would make the use of Salk vaccine impractical: the results would be so small in relation to cost and there is a crying need for money to combat diseases of far greater importance locally. An attenuated virus would be for many reasons more practicable. It has been suggested by several speakers that there may be a place for inactivated and a place for attenuated vaccines according to the epidemiologic and social background of the country concerned. About all these things there is really little place for argument. There are two or three important things we don't know and there is

virtual certainty that time—and hard work—will give us the answer. We do not know for how long a course of Salk vaccine will give protection against paralytic polio—with or without booster doses. That information will certainly come in the course of time. We do not know whether or not we can give attenuated viruses by mouth without too great risks from the consequences of reversion to greater neurotropism nor do we know how to ensure that they are not hyperattenuated thus losing their antigenic powers. Doubtless in appropriate

areas for study that knowledge will be acquired step by step in experiments of wider and wider scope. We have two potentially victorious weapons in the fight against poliomyelitis and we shall in due course learn how when and where we can use each of them and whether one is better than the other or they are complementary. Six years ago we weren't quite sure where we were going. Three years ago we thought we perceived a path. Now the road is becoming plainer and more hopeful all the time but it is still not yet a goal achieved. It is still a road.

## Summary on Care and Rehabilitation

DR SVEND CLEMMESSEN

It is difficult to give a summary on care and rehabilitation of patients severely stricken with poliomyelitis on the basis of the magnificent papers delivered at this Fourth International Poliomyelitis Conference. I feel humble in this privileged and honored position for which I am certainly not more qualified than any of the members of this Conference. Therefore I shall confine myself to speaking of subjective impressions from this past week and besides try to compare with the Proceedings of the Conferences in New York in 1948 in Copenhagen in 1951 and in Rome in 1954. I still hope that my personal experience will give some color and interest to the viewpoints maintained.

It is a characteristic feature of these poliomyelitis conferences that almost all the papers reveal some personal involvement with the subjects besides the scientific interest. Thus I shall never forget Dr Wilson's introduction here Wednesday in which he openly addressed us to balance our technique of lifesaving with our measures to make the life that is saved worth while and useful. "To give life to the lifetime saved. Here is more than Hippocrates claims!" An obligation of honor to the medical profession, a modern ethical medical obligation which will influence our daily work with other patients. In the future it will stand as an unavoidable obligation which we owe to our community and our patients. But how are we able to meet it?

The treatments with hemotherapeutics antibiotics and artificial respiration of various kinds have during the later epidemics saved so many patients with upper extremity disabilities that these problems have been forced upon us. It is a complicated problem to help these patients along the lines mentioned even if they have no respiratory insufficiency. Dr Landauer has told us of the most admirable American approach to the problems mentioned and Dr Nickel of the constant not finished evolution of reconstructive surgery combined with mechanical apparatus. Dr Afieldt has told us of the practical work of

the rehabilitation team in a respiratory and rehabilitation center and Dr Benton has given us a lot of practical experience on home care of patients with respiratory difficulties. Miss Locher speaking of vocational aspects has given us an instructive lecture in practically applied psychology and Dr Wendland has given us some mental and emotional aspects of poliomyelitis in a little masterpiece of a lecture which should be read by everybody who has to do with the chronically ill children or adults.

No wonder that we have been using the word care much more than the word treatment during our conferences!

Let us discuss statistics as they have been presented so clearly by Dr Lassen, Dr Hodess and Dr Ritchie Russell. Both the paralytic case fatality rate and the less significant total case fatality rate give us figures now so constant that in the future we shall be able to register the results obtained by vaccination. The later fate of patients chronically dependent upon artificial respiration and needs further study so that we can be able to prevent or at least postpone the complications. Further exchange of experiences might be useful here.

Statistics seem to show us the limit of what we are able to do with first-class respiratory aid and we all know that the methods of artificial respiration which have been developed for poliomyelitis are proving to be of great value in saving life in other diseases.

The long list of outstanding papers concerning artificial respiration prevents me from mentioning names of each of the authors. These papers all together form a veritable textbook of physiology and treatment of respiratory insufficiency. I am deeply impressed by this paramount review of applied physiology and technique which has been presented here as well as in the technical and scientific exhibits. The close and well-organized co-operation between the different respiratory centers, the co-operation with physiologists and the free international exchange



of experiences won have given the triumphs. On this background the unexpected important finding of the glossopharyngeal breathing technique is a very interesting and encouraging fact beneficial as it is to so many patients.

But how should we after all this be able to get further and still better results? The clinicians and physiologists have told us of the different complications which may occur respectively in patients with impairment of control of swallowing of food impairment of respiration and circulatory disturbances hyperventilation and hypoventilation acidosis and alkalosis and so on and how to distinguish between them. Still Dr Spencer states that it is entirely probable that the processes of the disease the physiologic effects of treatment procedures and the aggregations of complications have a variable timetable. This is the essence of the variability of the disease as the clinician views it. Practically the need for prompt syndromic anticipatory treatment is unquestioned. As Dr Wilson stated in 1953 and as it is now emphasized by Dr Ibsen the possible respiratory problem should be diagnosed before respiratory insufficiency occurs. Increased respiratory effort should be looked for rather than for the later occurring respiratory failure.

Dr Jungner has carried further the biochemical investigations and experiences of Bower *et al* and Astrup *et al* in his report.

The therapeutic range is narrow in the electrolyte disturbances. Both respiratory and metabolically conditioned acidosis or alkalosis occur and consequently determination of plasma bicarbonate alone often will not be sufficient. A possible renal acidosis should be reckoned with. The analyzing methods should be decisive in artificial respiration. The potassium dosage must be individualized. The risk of edema must not be underestimated and yet a high diuresis must be maintained. The raised urinary output of calcium and phosphate causing nephrocalcinosis must be counteracted from the start if the later serious complications are to be avoided. The genito-urinary and the gastro intestinal complications and their treatment have been described by Dr Sweet and Dr Neu respectively. The administration of anabolic steroids in cases of genito urinary complications is new and the treatment of gastro intestinal complications from

the start at the acute phase seems to be important.

If true anticipatory treatment is to be carried out in these patients we must go to the limits of what we are able to yield within application of physiology laboratory techniques and general medicine. To all this we must add our utmost within psychological treatment physical medicine and rehabilitation.

From conference to conference the solution of the difficult problems concerning scoliosis is progressive. Thus Dr Ottolenghi and Dr Bennett have now given us out of their deep experience in practice and literature not forgetting the valuable contributions of the discussant. The pioneer period within this subject has come to an end and these two papers together give us a comprehensive understanding of the subject where the individualized treatment of each patient is so decisive for the result obtained. Still I have a feeling that the final word concerning *the genesis of scoliosis* and *the causes of scoliosis* has not been said. By further study of the development of scoliosis we should be able to influence the prognosis further. The difficulty is that often the patients are not transferred to the scoliosis specialists until they have reached the late stages of scoliosis when the curves suddenly yield. Anticipatory treatment again and an attempt to influence the prognosis thereby!

Through Dr Buchthal's interesting paper concerning prognostic implication of electro myography we know that the fate of the single paretic muscle has been determined to a certain degree from the start. Also we know that the counteraction of contractures and disalignment of the lower extremities has been so successful that we obtain much better results now than we did only a few years ago and that we must not be passive and leave the muscles to spontaneous recovery.

The importance of the most meticulous approach to alignment of braces shoes and lower extremities during the growth period must be mentioned. Contractures always mean defeat.

I grew a little envious when I heard Dr Cooksey's paper concerning neuromuscular factors in rehabilitation in which he suddenly in a classic matter-of-fact way renews to us the theoretic background of our practical re-educational

tion of muscles and our counteraction of contractures. He builds his reasonings mainly upon the observation that it is the lack of proprioceptive stimuli from proprioceptors and muscle spindles which causes most of the wasting in the muscles. It is 50 years ago this year that Sherrington put up the concept of proprioceptors all these small end-organs which secure the whole co-operation within the body.

Through anticipatory treatment and close co-operation of all specialists we shall be able to get further in our care and rehabilitation of patients severely stricken with polio. In care and rehabilitation and in physical medicine we are accustomed to be in close co-operation with non-medical people.

As Clemenceau once said: War is too impor-

tant to be left to the generals, so the architect of our fight against polio Basil O'Connor, President of our International Poliomyelitis Congress recently paraphrased him and jokingly said that "science is too important to be left to the scientists." This is a friendly challenge but he himself has given us an example of functional idealism and co-operation. Through his work in the Red Cross and in the fight on polio he has been influencing our minds and has been the spokesman of private voluntary activity in close co-operation with public health and scientific experts all the time building on the humanitarian instinct of average people. Sir you have a unique faith in human resilience and peace! Let me say this before this audience on this special occasion.

## *Closing Remarks*

MR BASIL O'CONNOR

After those two excellent summaries and what has taken place this week one ought to be able to close this conference with a few words. In a sense that would be a little unfair. I would say briefly a few of the things that have impressed me as a layman during the four conferences we have had: one is the problem that has prevailed for many years, the belief that because a disease had a relatively low incidence it had a relatively low significance. That kind of thinking has been due to the fact that distinction was not made between the incidence of a disease and the value of the study of that disease. Conceivably less value might come from the study of a disease of high incidence than might come from a disease of relatively low incidence. These four International Conferences have demonstrated clearly that in the study of a relatively low incidence disease, namely poliomyelitis, a value can come in no sense comparable with the incidence of the disease itself.

Those of us who have followed the developments in what we call in my country after-effects care and research in after-effects realize that the whole activity has been one relating not only to disablement by a particular disease such as poliomyelitis but also one that has redounded to the benefit of all disabling diseases and such situations as respiratory conditions not necessarily connected with permanent disability.

Those of us who have followed the scientific research in the fields of the viruses come readily to the conclusion that we have practically covered the field down to almost the basic issues of life: the composition of the cells, the changes of cells and into the fields of genetics. All of that study while carried on to find a solution to the problem of poliomyelitis has a value far beyond a value that results in helpful solutions of other disease problems, diseases other than poliomyelitis. I hope that never again will scientists or laymen feel that the amount of money or the amount of time or the effort spent in studying a disease of low incidence has a value only com-

parable with the size of the incidence of that disease.

This conference has been increasingly more interesting than the third and the second and the first as it should be. An unusual amount of effort goes into these conferences in their scientific preparations. I speak now not of the practicalities connected with the arrangements of such a conference but in the preparation of the contents of the conference. After all a conference is to be judged by its content and its value if any to the public. The content of this conference was prepared carefully by a program committee of distinguished men to whom I wish to pay my respects and to offer your thanks as well as my own. The conference has been conducted intelligently and well. If there be some who feel that in all meetings international or national discussion seems to be increasingly discouraged we can say that we had a little more discussion at each of our conferences than we had at the preceding one and that we may hope that at future conferences we will have still more discussions.

We leave here feeling that real progress has been made in the possibility of preventing paralytic polio with an already existing killed virus vaccine. We are well aware of things that we would still like to have solved and explored further.

One of the questions about which we are not at all satisfied as yet is some method of determining and establishing the adequate potency of the vaccine so that what we know takes place now in some places can be eliminated. Only a layman would dare to make the statement that there is no reason why this kind of a vaccine should not be approximately 100 per cent effective if it is made properly which includes having the proper potency. We should not be satisfied with something like 75 or 80 per cent.

Another problem that we leave still in an unsatisfactory condition is the question of the application of this vaccine—to what extent it

should be applied throughout the world to whom it should be given where it should be given how it should be given and the proper spacing of it We still have the question as to the possibility of further types of poliomyelitis and as already has been said whether or not it is of another type If it does the same kind of damage we should feel the same kind of responsibility toward it Finally but not intending to include all of the things still left for consideration we would like to eliminate the monkey and get out of what we call in my country the monkey business

Since I know that you feel as we of the United States feel that the elimination of paralytic polio in one country only is not the goal that is sought I repeat that there is a likelihood that we will have eliminated paralytic polio in the United States by 1978 That is not the goal of any science and should not be that is not the goal of the International Congress The goal should be the elimination of paralytic polio

throughout the world If that is to take place over whatever reasonable period of time is necessary to accomplish it we might well consider the possibility of joint consideration of such problems from time to time by a small international group indicated by the International Congress In that way not only the solution of these problems might be expedited and their solution more readily agreed upon but also the value of an effective vaccine that can be given to the people of the world to the extent they wish to take it

As I close this conference I again think all of you for being here It is the people who come to these conferences that make the conference

Also I want to thank our Swiss friends and the officers of this conference and I want to thank particularly Dr C rasset for all the fine work the committee has done that made it possible for you and me to spend our time here profitably

I wish you a happy and safe journey home

## *Closing Comments of Chairman*

DR ROBERT DEBRE

After the words just pronounced by Mr Basil O'Connor it now falls upon the chairman of your final meeting to say a few words. I have little to say after hearing Mr O'Connor's statement.

The importance of the work reported and discussed here has already been mentioned. I should like to add that a victory has been achieved by establishing the value of the inactivated virus which was introduced by injection.

Here I should like to say a few words to our President Mr O'Connor and the National Foundation. I should like to express to them our sincere appreciation for the great assistance the Foundation has given us which has permitted us to obtain these results during these great campaigns which were carried out in the United States of America. The participation of the population and of the physicians was obtained in an experiment which is unique in its times and has made it possible to affirm the value of the vaccine prepared by Jonas Salk. The immunity it confers has been demonstrated in the course of this meeting. Those who at the beginning were disturbed by incidents with which you undoubtedly are familiar may today recall what happened at the time of the Lubeck incident, when the error of a physician disturbed minds regarding another vaccine. Today we all know the value of the inactivated Salk vaccine and we know about the immunity it confers. We know that it develops antibodies which can protect the nervous system but we also know that we still have much to learn. We need to know the percentage of subjects that have been protected, we need to know the degree of protection that is afforded by this vaccine and we need to know about the duration of the protection.

Lastly the technique of manufacture is never completed and you know here again that a certain number of authors such as Dr Lepine have insisted on a certain number of questions. Acknowledging the success which has been

obtained by the injection of inactivated virus I should like to pay tribute here also to those authors who have worked on the problem of the attenuated virus. It is important to remind you here once again of the interest of the infection which is not apparent which is nonpathogenic and which imitates the natural infection. The interest resides in the studies performed by men like Koprowski and Sabin which show the great variations of the pathogenic power of the virus resulting from mutations in the human body, the interference which may result and the competition among the viruses in the human body and lastly a great and major interest may reside in tissue immunity which is different from that mediated by antibodies.

As the problem of vaccination has advanced and progressed the problems of public health which result from poliomyelitis as Mr O'Connor insisted are far from being completely solved. Its epidemiology is still obscure. How does the germ spread? How does it go from one man to another man? How with an infection which is so widespread which is so benign is it possible that some unfortunate human beings become victims of neurologic complications? Lastly as far as public health is concerned another problem arises again and that is the problem of inaccurate diagnosis. It concerns the distinction between diseases which are due to a polio virus and those caused by other viruses to which Dr Andrewes referred a few moments ago. The Coxsackie viruses the ECHO viruses all of these are widespread and some are involved in certain morbid disorders such as the Bornholm disease. But it is impossible as yet to define the exact role that these particular types of viruses play nor can we determine anything so far about effects they may have in certain cases of paralysis. Thus shows you the tremendous field that is still open to the workers over the world today.

But as Mr O'Connor has said it is possible that from polio we have turned today to a study

of a biologic and fundamental behavior of viruses the phenomena of biosynthesis how it is that an infecting particle actually establishes itself with its acid and its protein and how this little particle infects a new cell. In the course of this conference we have heard much about the common properties of various viruses far removed from one another such as tobacco mosaic virus the potato virus and all the various enteric viruses including polio virus itself. Here chemistry and the chemistry of biology have certainly become the subject of this conference in addition to polio. Thus we have expanded in a very unexpected manner the field of our knowledge and research.

I will not speak about rehabilitation nor say anything about the assistance to be given to respiration. Of course the truth is that a good pupil can follow only one class at a time and therefore he cannot be considered guilty for not being at two places at the same time.

Fortunately Dr. Lemmesen has summed up for those who followed the study on viruses precisely what had been said in connection with respiration and also in connection with motor trouble and disorders.

I have spoken too much at length and I ask your forgiveness. As the Chairman of your last session it is my pleasure and privilege at this time to pay tribute once again and never enough to our hosts in Geneva to Professor Casset and his assistants to the professors of the Faculty of Medicine in Geneva the authorities of this canton of the city of this small and noble republic. It is up to me also to thank on your behalf the National Foundation for the great effort that it has made for the preparation the organization in fact the success of this conference and at the same time to ask that Foundation some day to have the pleasure of meeting with them again. The last session of the Fourth Polio-mis Conference is hereby closed.



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